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STAIN TECHNOLOGY

VOLUME II

JANUARY, 1936

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NUMBER 1

AN ENLARGEMENT OF STAIN TECHNOLOGY

It is a happy coincidence that at the end of STAIN TECHNOLOGY's first decade we are able to announce, after a year's preparation, the expansion of the section entitled "LABORATORY HINTS FROM THE LITERATURE" into a much larger department under the charge of one man as abstract editor. The enlargement of this section is to be such as to constitute virtually a new feature for the journal. This idea has been under consideration for some time and announcement of the plans were made in SCIENCE early this year. The response to this announcement was very much greater than we anticipated. We have not only found the Commission members ready with enthusiastic support of the plan, but have received so many offers for assistance from those not previously associated with the Commission that this new undertaking now has a fair-sized organization of abstracters behind it. The editors hereby extend their thanks to all those who have offered to assist. The result of this collaboration should certainly be a better and larger department of "LABORATORY HINTS". It is hoped that this venture will find adequate reception among our readers and biologists at large.

The policy in this section of the journal has always been to furnish the readers with abstracts that are *usable* in the laboratory, and this same plan will be followed in the future whenever possible. Accordingly, the abstracts published in STAIN TECHNOLOGY ordinarily give the technic in sufficient detail so that it can be followed without consulting the original article. The exceptions to this rule are those papers where a great variety of procedures is described, and those which deal with dyes in general rather than with technic. Altho our policy will be followed wherever possible, exceptions may be more frequent in the future than in the past, because the field covered is no longer to be limited to a few subjects. We plan, in fact, to include any line that might be of interest to users of the microscope in general. Naturally, special attention will be paid in the future, as it has always been in the past, to dyes in all their biological applications.

At the present time this service covers some 160 journals issued in 21 different countries. These journals have been assigned to those who have offered to help in the work; and the large number of abstracts secured for the present number shows that the abstracters are actually cooperating. The present number contains 60 abstracts and represents the work of 10 abstracters. We wish here to express our appreciation not only to the abstracters who have actually contributed to this number, but also to all the other collaborators (there are nearly 50 in all) whose assistance has made it possible to undertake the project.

—J. A. DE TOMASI, *Abstract Editor*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the October number of this journal.

STAINS CERTIFIED SEPTEMBER 1, 1935 TO NOV. 30, 1935*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Crystal violet	LC 9	92% ₀	As bacteriological, histological and cytological stain	Sept. 5, 1935
Safranin O	LS 5	87% ₀	As cytological and histological stain	Sept. 10, 1935
Indigo carmine	NI 5	91% ₀	As histological stain	Oct. 21, 1935
Basic fuchsin	CF 15	91% ₀	For general staining and in the Endo medium	Nov. 21, 1935

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

SUGGESTED COUNTERSTAINS FOR DAVENPORT REDUCED SILVER PREPARATIONS OF PERIPHERAL NERVES

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ABSTRACT.—Peripheral nerves which have been fixed in a mixture of formaldehyde and acetic acid and stained according to the method of Davenport can be successfully counterstained for demonstration of myelin sheaths and stroma. After mounted sections have been silvered, reduced and toned, the coating of nitrocellulose is removed by passing thru two changes of acetone. Following brief washes in 100, 95, 85 and 75% alcohols they are stained in an acidified aqueous solution of azo carmine for 30 to 60 minutes. Excess azo carmine is extracted with anilin alcohol followed by acetic alcohol after which the sections are mordanted for 15 to 60 minutes in a 5% aqueous solution of phosphotungstic acid. Without washing they are transferred to a stain mixture of either anilin blue and orange G (acidified) or light green and orange G (acidified) where they remain from 1 to 5 hours. After destaining in 95% alcohol and dehydration in absolute alcohol the sections are mounted in dammar. Result: axons stain black; sheath and fibroblast nuclei, red; myelin sheaths, orange; and connective tissue, blue or green. When the counterstains are applied to ganglia, cytological details of individual cells are demonstrated.

INTRODUCTION

When most silver methods are applied to peripheral nerves, axis cylinders stain selectively; consequently, sheaths and supporting tissues are incompletely demonstrated. Large myelinated fibers can be identified readily in such preparations, since the big axons are surrounded by large clear areas (unstained sheaths). Small myelinated fibers, however, can be recognized less easily, since the axons are reduced in diameter and the enveloping clear zones are decreased proportionately. In fact, differentiation between the smallest myelinated and the largest unmyelinated fibers frequently is impossible. To add to the confusion, clumps of chromatin in sheath nuclei occasionally simulate unmyelinated axons. These inadequacies of silver when used alone suggest the need for counterstains which will show clearly both sheath and stroma, and thus aid in separation and classification of fibers according to type. Helpful in this direction are variations of Heidenhain's modification of the Mallory triple

stain as described by Romeis¹ which we have used in conjunction with the Davenport² silver technic.

A. Stock Solutions of Stains

STAINS AND REAGENTS EMPLOYED

1. AZOCARMINE MIXTURE

Azocarmine (Grubler, dye content not stated).	1.0 g.
Glacial acetic acid (Merck, C. P. 99%)	4.0 cc.
Distilled water.	100.0 cc.

2. ANILIN BLUE ORANGE G MIXTURE

Anilin blue (Grubler, dye content not stated)	0.5 g.
Orange G (Coleman and Bell Co., Certified 82%, representing 1.64 g. actual dye)	2.0 g.
Glacial acetic acid (Merck, C. P. 99%)	1.0 cc.
Distilled water	100.0 cc.

3. LIGHT GREEN ORANGE G MIXTURE

Light green (Eberbach & Sons Co., dye content not stated)	0.5 g.
Orange G (Coleman and Bell Co., Certified 82%, representing 1.64 g. actual dye)	2.0 g.
Glacial acetic acid (Merck, C. P. 99%)	1.0 cc.
Distilled water	100.0 cc.

4. NOTE: Stock solution No. 1 is used full strength. 10 cc. of stock solution No. 2 or No. 3 are diluted with 40 cc. of distilled water before using.

B. Stock Reagents

1. Acetone (Baker's analyzed, C. P.)
2. Anilin oil (Coleman and Bell Co., C. P.)
3. Dammar (Coleman and Bell Co.)
4. Formaldehyde (Merck's blue label, 37%)
5. Glacial acetic acid (Merck, C. P., 99%)
6. Gold chloride (Merck, C. P.)
7. Low viscosity nitrocellulose (Hercules Powder Co.)
8. Paraffin (Leitz, white filtered, M. P. 60-62° F.)
9. Petroleum ether (Coleman and Bell Co., b. p. 40-60° C.)
10. Phosphotungstic acid (Merck)
11. Pyrogalllic acid (Mallinckrodt, C. P.)
12. Silver nitrate (Merck's reagent grade or Coleman and Bell Co., C. P.)
13. Sodium thiosulfate
14. Xylol (Coleman and Bell Co.)

¹Romeis, B. *Taschenbuch der Mikroskopischen Technik*. R. Oldenbourg, Munchen. pp. 365-6. 1928.

²Davenport, H. A. Staining nerve fibers in mounted sections with alcoholic silver nitrate solution. *Arch. Neur. and Psych.*, 24, 690-5. 1930.

PROCEDURE

A. Technic of Staining with Silver.—Stretches of the glossopharyngeal and vagus nerves of the cat were fixed in a mixture of formaldehyde and acetic acid (formaldehyde 25 cc., glacial acetic acid 5 cc., and distilled water 70 cc.), dehydrated in graded ethyl alcohols, cleared in xylol and embedded in paraffin. Sections 5 microns thick were silvered, reduced and toned according to the method described by Davenport.³

After the sections had been toned in gold chloride, treated for 5 minutes with a 5% aqueous solution of sodium thiosulfate and thoroly washed in tap water, the coating of nitrocellulose was removed by passing the slide with constant agitation thru two changes of acetone. Finally, the tissues were prepared for the counterstain by brief washes in 100, 95, 85, and 75% alcohols.

B. Technic of Counterstaining.—(All procedures at room temperature.) 1) From 75% alcohol to azocarmine; 2) Azocarmine, *30 minutes to one hour*; 3) Destain in anilin alcohol (95% alcohol 1000 cc., anilin oil 1 cc.) *until nuclei are clearly differentiated*. (Process may be facilitated by passing slide back and forth from 95% to 75% alcohol.) 4) Acetic alcohol (95% alcohol 1000 cc., and glacial acetic acid 1 cc.), *1 to 5 minutes*; 5) Phosphotungstic acid (5% aqueous solution), *15 minutes to 1 hour*; 6) Either anilin blue orange G mixture, or light green orange G mixture, *1 to 5 hours*; 7) Destain in 95% alcohol, *until tissue elements (other than axons) are clearly differentiated*. (Process may be facilitated by passing slide back and forth from 95% to 75% alcohol.) 8) Absolute alcohol, *two changes, 1 to 3 minutes each*; 9) Xylol, *four changes, several minutes each*; 10) Mount in dammar which has been extracted with cold petroleum ether, evaporated to dryness, and dissolved in xylol.

RESULTS

Axis cylinders are stained black, sheath and fibroblast nuclei red; myelin sheaths orange; and connective tissue blue or green, the latter depending upon whether anilin blue or light green is used (Figs. 1 and 2). If too much azocarmine is removed during the destaining process, the nuclei and the sheaths will be yellow instead of red and orange, respectively.

³Thru the courtesy of Dr. Davenport we have employed his method with certain modifications which he has suggested (see footnote 2) and which we have found advantageous. These alterations of the original technic are: a) the use of a 20% instead of a 10% alcoholic silver bath; b) an 8% instead of a 5% concentration of pyrogalllic acid; and c) a 4% solution of nitrocellulose instead of a 2% solution of celloidin.

PLATE 1. EXPLANATION OF FIGURES

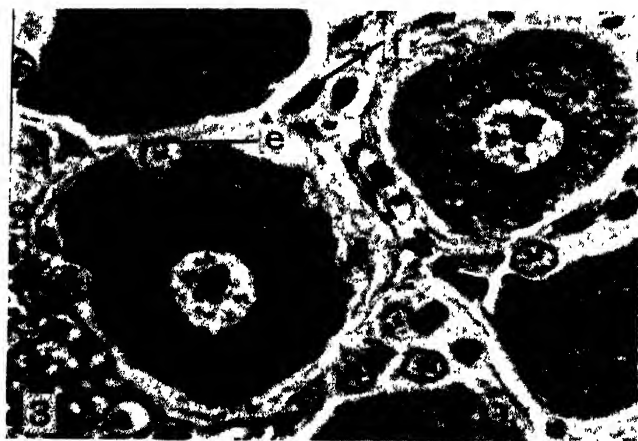
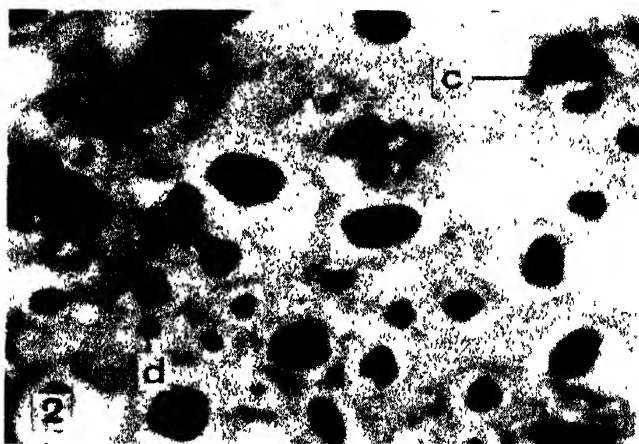
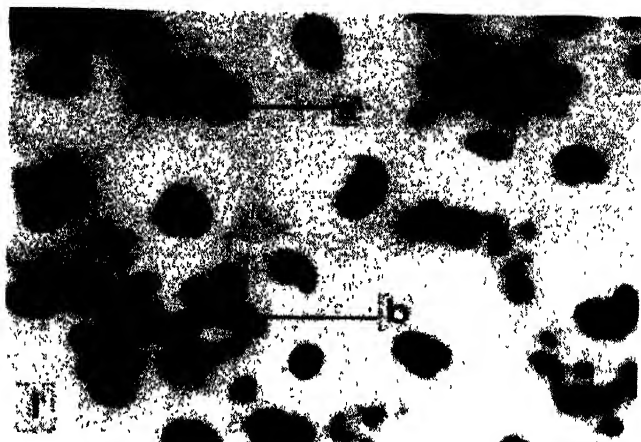
Photomicrographs of sections of the vagus and glossopharyngeal nerves of the cat.

1. A cross section of the vagus below the nodose ganglion (Davenport silver; azo-carmin, anilin blue and orange G counterstain; $\times 3000$). Note the clump of unmyelinated fibers (a). In full color the tissue core of (a) is blue or purple whereas the core of (b), a nucleus, is red; the myelin sheaths are orange, while the intervening collagenous tissues are blue. It should be observed that the groups of black unmyelinated fibers are embedded in collagenous tissue.

2. A cross section of the glossopharyngeal below the petrous ganglion (Davenport silver; azocarmine, light green and orange G counterstain; $\times 3200$). All axons in the field are myelinated. Observe the range in size of the axons. Each is surrounded by an orange myelin sheath (light gray and white in photograph) and is separated from adjacent fibers by green collagenous tissue (dark gray in photograph). In contrast to the vagus nerve (Fig. 1) there are no clumps of unmyelinated fibers. Note the sheath nuclei, (c, d). These stain red and each is related to a small myelinated fiber.

3. A cross section thru the nodose ganglion of the vagus (Davenport silver; azo-carmin, anilin blue and orange G counterstain; $\times 800$). Attention is directed to the sharpness with which the neuro-fibrillae and nucleoli of the cells are outlined (particularly in the cell at the upper right of the photograph). Note the fibroblast nucleus (f) of the connective tissue capsule of the ganglion cell and the nucleus (e) of the sheath of Schwann cell. Several myelinated fibers are clearly shown between the two nucleated cells.

SILVER PREPARATIONS OF PERIPHERAL NERVES



When the counterstain is applied to ganglia, myelin sheaths of nerve fibers, sheath nuclei and stroma are stained as above. In addition, the finer cytological details of the individual ganglion cells are demonstrated, the neurofibrillae of the cells being black, the hyaloplasm orange or yellow, and the nucleolus red. Likewise, the nuclei of the connective tissue capsule of each cell and those of the Schwann cell capsule are fully differentiated (Fig. 3). Since by the methods outlined nucleoli of cells can be stained, one should be able to make counts of ganglion cells in nerves that have been silvered previously for axon studies.

LACTO-PHENOL PREPARATIONS

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ABSTRACT.—More or less permanent mounts of fungi, algae, root tips, epidermis, germinating spores, and other small objects may be made readily by transferring the material to Amann's lacto-phenol containing anilin blue, W. S. or acid fuchsin, used singly or mixed. The addition of 20 to 25% of glacial acetic acid to these mixtures is frequently advantageous; or material may be stained with various dyes—acid fuchsin, anilin blue, W. S. (cotton blue), rose bengal, phloxine, hematoxylin—in aqueous solutions containing 5% of phenol, and then mounted in lacto-phenol, 50% glycerin or phenol-glycerin, depending on the dye used. The phenol solutions of acid fuchsin and anilin blue are acidified with acetic acid and those of rose bengal and phloxine are made slightly alkaline with ammonium hydroxide. The addition of ferric chloride to acid fuchsin or acidified hematoxylin may improve staining. Fixation may be preferable but may be omitted, especially with fungi. Formulae for the mounting media and ten staining mixtures are given.

Lacto-phenol-dye mixtures have long been used for temporary mounts, especially of fungi. Much less cotton blue than is recommended by Linder¹ is often preferable. Substitution of another acid dye (especially acid fuchsin) for cotton blue (syn. anilin blue, W. S.), or using two dyes mixed together is frequently advantageous. The addition of 20 to 25% of glacial acetic acid makes staining more rapid and generally not as deep as without the acid. Fungi transferred to this mounting medium may be studied immediately or the preparations may be kept for a year or longer. In making such mounts many variations are possible. The formulae for the various stains and mounting media are given below. The following dyes were used: Acid fuchsin, cotton blue (anilin blue, W. S.) and so-called "Magdala red", Coleman and Bell (the last named being actually phloxine); rose bengal, Grubler; hematoxylin, Coleman and Bell. C. P., Certification No. FH-5.

FORMULAE

1. a. *Lacto-phenol*, Amann's: phenol, 20 g. or cc.; lactic acid, 20 cc.; glycerin, 40 cc.; water, 20 cc.
- b. *Phenol-glycerin*: phenol, 20 g. or cc.; glycerin, 40 cc.; water, 40 cc.

¹Linder, David H. An ideal mounting medium for mycologists. *Science*, 70, 430. 1929.

2. *Lacto-phenol-dye (1% aqueous) mixtures.*

- a. Lacto-phenol, 100 cc.; cotton blue, 1 to 5 cc.; glacial acetic acid, 0 or 20 cc.
- b. Lacto-phenol, 100 cc.; acid fuchsin, 1 to 5 cc.; glacial acetic acid, 0 or 20 cc.
- c. Lacto-phenol, 100 cc.; acid fuchsin and anilin blue, W. S., 1 to 3 cc. each; glacial acetic acid, 0 or 20 cc.

For algae, fungi, root tips, epidermis, *Elodea* leaves, etc. Mount in lacto-phenol or lacto-phenol plus 20% of glacial acetic acid.

3. *Phenol (5% aqueous) dye (1% aqueous) mixtures.*

- a. Phenol, 15 cc.; anilin blue, W. S., 0.5 to 1 cc.; glacial acetic acid, 4 cc.
- b. Phenol, 15 cc.; acid fuchsin, 0.5 to 1 cc.; glacial acetic acid, 4 cc.
- c. Phenol, 15 cc.; acid fuchsin, 0.5 to 1 cc.; glacial acetic acid, 4 cc.; ferric chloride, (30% aqueous) 2 cc.
- d. Phenol, 20 cc.; phloxine, 10 cc.; NH_4OH (conc.) to slight alkalinity.
- e. Phenol, 20 cc.; rose bengal, 10 cc.; NH_4OH (conc.) to slight alkalinity.

For algae use *a* to *e*, for fungi *a* to *c*, and for root tips, epidermis or *Elodea* leaves, *b* or *c*. Mount *a* to *c* in lacto-phenol plus 20% glacial acetic acid; *d* and *e* in 50% glycerin.

4. *Carbol-hematoxylin.*

- a. Water, 95 cc.; hematoxylin, 1 g.; phenol, 5 g. Add the hematoxylin to the water, bring slowly to boiling with occasional shaking; add the phenol and cool.²
- b. Carbol-hematoxylin, 20 cc.; HCl (conc.), 1 cc.
- c. Carbol-hematoxylin, 20 cc.; HCl (conc.), 1 cc.; FeCl_3 (30% aqueous), 0.5 cc.

For algae, some fungi, epidermis, root tips, *Elodea* leaves, pollen mother cells, etc. Mount in lacto-phenol made slightly alkaline with concentrated NH_4OH , or in phenol-glycerin (1 *b*).

PROCEDURES WITH LACTO-PHENOL-DYE (2) OR PHENOL-DYE (3) MIXTURES

Material may be mounted and stained without fixing, but fixation for 1 to 12 hours in formol-acetic (formaldehyde, 10 cc.; glacial acetic acid, 5 cc.; water, 85 cc.) is generally preferable, excepting for most fungi. After fixing wash for a short time in water and transfer the material directly to the dye mixture (2 or 3) in which staining will be rapid. After staining lacto-phenol-dye (2) mounts will often be satisfactory for immediate examination. If staining is deep or one of the phenol-dye (3) mixtures was used, replace it with the appropriate medium, added at one edge of the cover glass as the stain is with-

²Shortt, H. E. Rapid method for Heidenhain stain. *Indian J. Med. Re.* 1923. Cited from Lee's *Microtomist's Vade-Mecum*, 9th Ed., p. 162. 1928.

drawn from the opposite edge; or remove the cover glass, drain, and apply the medium.

Algae such as *Spirogyra* when stained with acid fuchsin, phloxine, or rose bengal show nuclei, chromatophores, pyrenoids (surrounded by shining starch granules) and protoplasmic strands clearly stained. Root tips, especially if stained with acid fuchsin and cotton blue (2 c.), show the embryonic region clearly differentiated from the older portion with the nuclei well stained. Leaves of *Elodea* (water-weed) stain slowly (6 to 24 hours). Acid fuchsin is recommended for them. The epidermis of *Tradescantia* spp. (spiderwort) or of *Oenothera lamarckiana* (day-flower) is excellent for mounts. It may be removed from the leaves in pieces a centimeter square. If only a little dye is used the nuclei will be deeply colored in lightly stained cells.

PROCEDURES WITH CARBOL-HEMATOXYLIN (4)

Staining with hematoxylin may be preferable to that with other dyes, especially for nuclei. While it may be omitted, fixing for fifteen minutes to several hours in a chrom-acetic solution (chromic acid, 0.7 g.; glacial acetic acid, 0.5 cc.; water, 100 cc.) gives better results. After fixation wash at least a short time in water and transfer the material to six drops of carbol-hematoxylin *freshly* mixed with one drop of 30% aqueous FeCl_3 , the mixing being done with a glass rod on the slide; or else transfer to acidulated carbol-hematoxylin containing FeCl_3 (4 c.). Stain 1 to 3 minutes, wash in water, and mount in slightly alkaline lacto-phenol or in phenol-glycerin. (Slides prepared in this way are usually best after a day and sometimes fade, but may be restained.) Instead of mixing the carbol-hematoxylin with the FeCl_3 the latter may be added to the fixing solution (1 cc. to 10 to 15 cc.). Then stain in carbol-hematoxylin or in carbol-hematoxylin acidulated with HCl (4 b.) and mount as above.

The mixture of carbol-hematoxylin and ferric chloride is stable for only a few minutes, so should be prepared *just before using*. It stains deeply and quickly. If staining is too deep, a brief washing in 2 to 4% FeCl_3 will reduce it.

COMPARATIVE STUDY OF DEHYDRATION

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Columbus, Ohio*

ABSTRACT.—The paraffin method has frequently been criticised because of its hardening and shrinking effect on tissue. The author believes this distortion is due to the dehydration and not to the immersion in melted paraffin. An experimentally controlled series of various tissues was dehydrated in different dehydrating reagents, dioxan, iso-butyl alcohol, and ethyl alcohol with chloroform. Except for the dehydration, the tissues were treated identically. In every case, dioxan proved to be a better dehydrating reagent with less shrinkage and brittleness than any of the others. Ethyl alcohol with chloroform produced the greatest degree of distortion.

INTRODUCTION

Within recent years, numerous suggestions have been made concerning the ethyl-alcohol-xylene series in the paraffin method. The chief criticism of this series has been of its hardening effect on the tissues. Hence, the search for some reagent which would satisfactorily replace the ethyl-alcohol-xylene series without its disadvantages.

Larband (1921) proposed the mixture of ethyl and *n*-butyl alcohol, which later was recommended by Zirkle (1930). Painter (1924) substituted anilin oil for the higher concentrations of ethyl alcohol. Sheridan (1929) proposed the use of *n*-propyl alcohol; Bradbury (1931) the use of iso-propyl. Margolena (1932) and Stiles (1934) obtained excellent results with animal and insect tissues by using *n*-butyl alcohol, but Smith (1931) had little success with it. Hewitt (1931) wrote quite favorably of iso-butyl alcohol. Sass (1932) recommended the substitution of ethyl alcohol with acetone.

Graupner and Weisberger (1931) completely omitted the alcohol series by removing tissues directly from water, or fixative, into dioxan, a reagent which is miscible with water or alcohol and is a solvent of paraffin. The Turttox News (1934) reported satisfactory results with dioxan, altho their limited experimentation was restricted to Zenker-fixed tissues.

Walls (1932) from his success with the hot celloidin technic, postulated that it is the hot paraffin, and not the heat "per se", which shrank and hardened the tissues, but Stiles suggested that it may be a matter of dehydration and clearing and not of paraffin impregnation. McClung (1929) omitted the soft paraffin in the classical alco-

¹New located at The Biological Supply House, 761 E. 69 Pl., Chicago, Ill.

hol xylene method and passed directly from xylene into hard paraffin, thus shortening the time in melted paraffin to a minimum.

Apparently, no one has reported any extensive comparative study of this problem, or considered the question of relationship between fixation and the subsequent treatment.

Formaldehyde is generally regarded as a rapid penetrator, but as taking a long time to exert its full effects. In the ordinary short fixation, great shrinkage often takes place during dehydration. This may be due to the shrinking of incompletely solidified protein compounds by ethyl alcohol, or to the effect of formaldehyde upon the lipides of cell membranes.

Bouin's fluid produces very little shrinkage and distortion in the cellular elements. Furthermore, the tissues maintain a sufficiently soft consistency for easy sectioning. Zenker's fluid, or modifications, constitute the most common fixatives for cytological detail, altho the tissue is often rendered very hard, brittle, and crumbly during after treatment.

Thus, we have represented by the three most common laboratory fixatives, three conditions of tissues; namely, soft consistency at first, but usually shrinking and hardening during dehydration; soft consistency thruout; and a hard, brittle condition from the start. Is there any one method which would satisfactorily prepare all three types of tissues for easy sectioning?

To answer this question, the comparative study of various dehydrating methods was started. The selected methods were fast dioxan, slow dioxan, iso-butyl alcohol (representing the higher alcohols), and the classical ethyl-alcohol-xylene technic. Chloroform, however, was substituted for xylene, as by previous experience chloroform was found to produce less brittleness in tissues and is more easily removed than xylene.

Dioxan (diethyl oxide) is a colorless liquid with a faint odor, a boiling point of 101° C. and melting point about 8° C. Its specific gravity is near that of water (1.0418) and its volatility rather high. It is miscible in all proportions with water and with alcohol. It dissolves paraffin slightly when cold, but quite readily when heated.

Iso-butyl alcohol is a straw colored liquid with a distinct pungent odor, a boiling point of 107° C., a specific gravity below that of water (0.810) and of a rather high volatility. It is miscible with water to the same extent as ethyl acetate and is a solvent of paraffin, particularly when heated. It is toxic internally and may produce headaches when its vapors are inhaled.

METHODS

Animal tissue, such as liver, striated muscle, thyroid, and intestine, which usually present technical difficulties, were fixed for 24 hours in 10% formalin, Bouin and Zenker formol. Four tissue blocks from the same organ and the same animal were placed in each fixative. The blocks measured $1 \times 1 \times 0.5$ cm., the average size for histological and pathological preparations. A human colloidal tumor was selected for the thyroid material, while the rest of the tissue was of white rat. A colloidal tumor probably presents more technical difficulty than any other tissue, the colloid being particularly susceptible to hardening, fragmentation and brittleness. After fixation, the tissues were washed in running water, 6 hours for formalin material, 24 hours for Zenker formol, while the Bouin material was placed directly into 70% ethyl alcohol.

The four blocks were then separated, one for each dehydrating process, dehydrated, embedded and sectioned at 10 microns. Representative sections at different levels of each block were mounted and stained simultaneously in the same staining tray with Harris' hematoxylin and eosin. Thus, except for the dehydration process, each section received identical treatment.

The detailed methods are as follows:

A. Fast dioxan: 1) fixation; 2) wash in running water; 3) dioxan, 4 to 6 hours; 4) saturated solution rubber-paraffin¹ in dioxan in oven, overnight; 5) rubber-paraffin, 2 hours; 6) rubber-paraffin, 2 hours; 7) embed.

B. Slow dioxan: 3) 2 parts water + 1 part dioxan, 2 hours; 4) 1 part water + 2 parts dioxan, 2 hours; 5) dioxan, 2 hours; 6) saturated solution rubber-paraffin in dioxan in oven, overnight; 7) rubber-paraffin, 2 hours; 8) rubber-paraffin, 2 hours; 9) embed.

C. Iso-butyl alcohol: 3) 70% alcohol, 6 to 12 hours; 4) 95% alcohol, overnight; 5) iso-butyl alcohol, 2 hours; 6) iso-butyl alcohol, 2 hours; 7) rubber-paraffin, 2 hours; 8) rubber-paraffin, 2 hours; 9) embed.

D. Ethyl-alcohol-chloroform: 3) 70% alcohol, 6 hours; 4) 95% alcohol, overnight; 5) absolute alcohol, 4 hours; 6) absolute alco-

¹The formula for the rubber-paraffin is as follows:

A) Stock rubber-paraffin, a saturated solution of crude rubber in 53-55° paraffin.

B) Paraffin, 53-55°, 720 g.; stock rubber A, 140 g. in winter or 160 g. in summer; bayberry wax, 80 g.; anilin oil, 5 cc.

hol, 4 hours; 7) chloroform, 4 to 6 hours; 8) chloroform-paraffin, overnight; 9) soft paraffin, 1 hour; 10) soft paraffin, 1 hour; 11) rubber-paraffin, 1 hour; 12) rubber-paraffin, 1 hour; 13) embed.

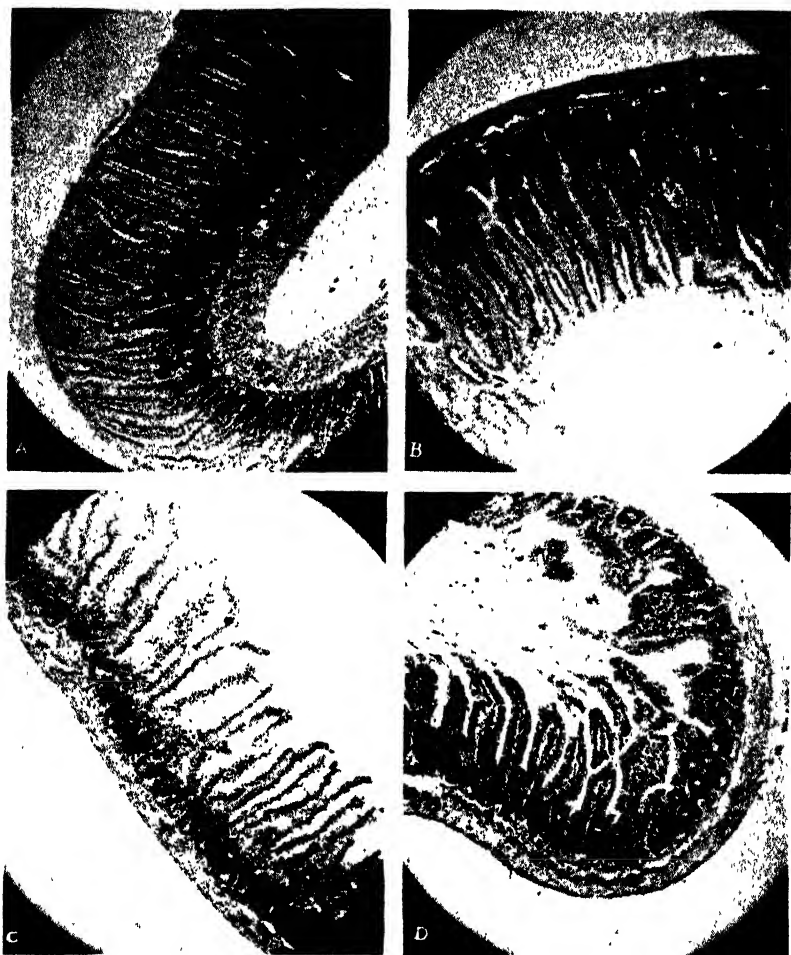


Plate I. Small intestine of white rat, fixed 24 hours in Bouin's fixative, adjacent tissue blocks. The tissue was placed directly into 70% ethyl alcohol after fixation. The blocks were then separated and passed thru the dehydrating reagents; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

The Bouin material was placed directly into 70% ethyl alcohol and the dioxan dilutions were made with alcohol instead of water.

DISCUSSION

While dioxan does not completely fulfill the dreams of a microscopist for a perfect dehydrating reagent, yet it is definitely superior to any present known substance (Chart 1). Regardless of the fixation, tissues retained a fairly soft consistency with a minimum of brittle-

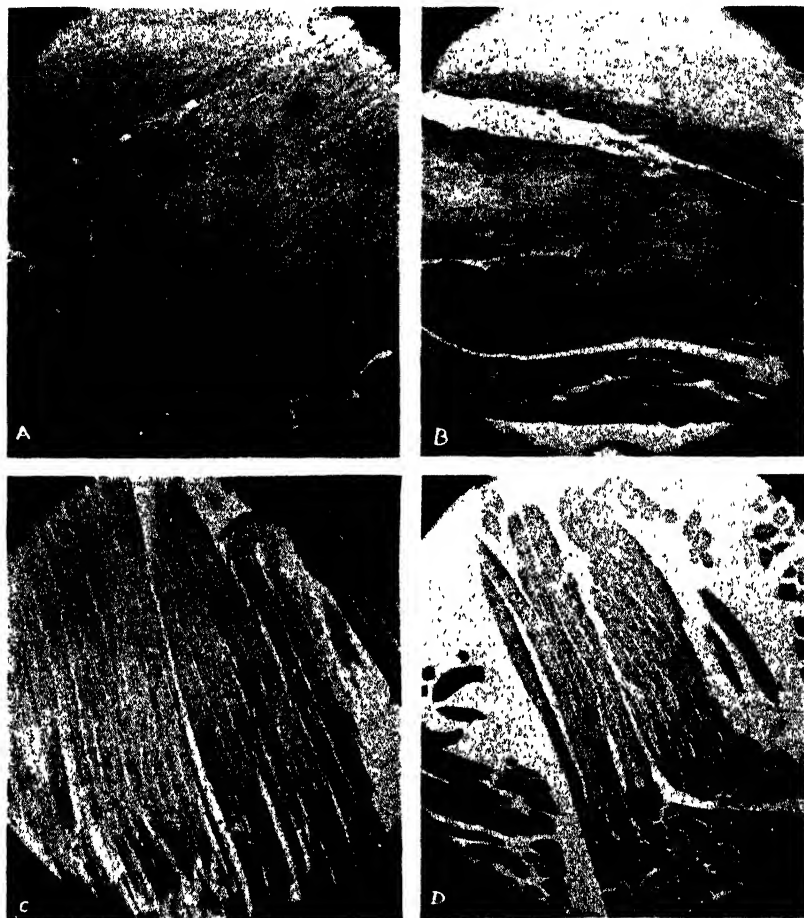


Plate II. Muscle of white rat, fixed 24 hours in 10% formalin, and washed 6 hours in running water, adjacent tissue blocks. The tissue blocks were separated and passed thru the dehydrating reagents; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

ness and distortion. The experimental use of different fixatives, adjacent tissue blocks from the same organ and same animal, and simultaneous staining, reduced fixation and tissue variation to a

TISSUE	FIXATIVE	DEHYDRATING REAGENTS	IMPREGNATION			SECTIONS				MICRO- EXAM.	
			GOOD	POOR	INCOMPLETE	GOOD	POOR	SHATTERS	RASPS	SHRINKAGE	SHATTERING
INTESTINE	FORMALIN	FD	+			+				++	
		SD	+			+				++	
		IB	+			+				+++	
		AC	+			+				+++	
	BOUIN	FD	+			+				+	
		SD	+			+				+	
		IB	+			+				+	
		AC	+			+				++	+
	ZENKER- FORMOL	FD	+			+				+	
		SD	+			+				+	
		IB			+	+				+	+
		AC			+	+				++	+
MUSCLE	FORMALIN	FD	+			+		SL			
		SD	+					+			
		IB		+	+		+	+	+	+	+
		AC	+				+	++	+	++	+
	BOUIN	FD	+			+					
		SD	+			+					
		IB	+		+	+				+	
		AC	+			+				+	+
	ZENKER- FORMOL	FD	+				+			+	
		SD	+					SL		+	
		IB			+		+	+	+	++	+
		AC	+				+	++	+	++	++
LIVER	FORMALIN	FD	+			+				+	+
		SD	+			+				+	
		IB			+					+	
		AC	+			+		+	+	++	+
	BOUIN	FD	+			+					
		SD	+			+					
		IB			+	+				+	
		AC	+			+				++	
	ZENKER- FORMOL	FD	+			+				+	
		SD	+			+				+	
		IB	+			+				+	+
		AC	+			+			+	++	+
THYROID COLLOIDAL TUMOR	FORMALIN	FD	+		+	+			+	+	+
		SD	+			+				+	+
		IB	+		+	+				++	++
		AC		+			+	+	+	++	+++
	BOUIN	FD	+		+	+			+	++	+
		SD	+			+				++	
		IB	+		+	+				++	++
		AC	+		+	+		+	+	++	+++
	ZENKER- FORMOL	FD	+			+				+	+
		SD	+			+				+	
		IB	+			+				++	SL
		AC	+			+		+	+	++	+++

Chart 1. Chart of experimentally controlled series of animal tissue. The tissues in all cases received identical treatment except for the dehydration process. Representative 10μ sections were made at different levels thruout the block and were stained by the routine hematoxylin and eosin method.

Abbreviations: fd. fast dioxan; sd. slow dioxan; ib. iso-butyl alcohol; ac. ethyl-alcohol-chloroform; sl. slightly.

minimum. Thus with all extraneous factors controlled, one feels fairly assured that the differences produced are the results of dehydration. As a check upon this assumption, frozen sections of the fixed tissue were made, thus presenting as nearly a normal condition of fixation as possible.

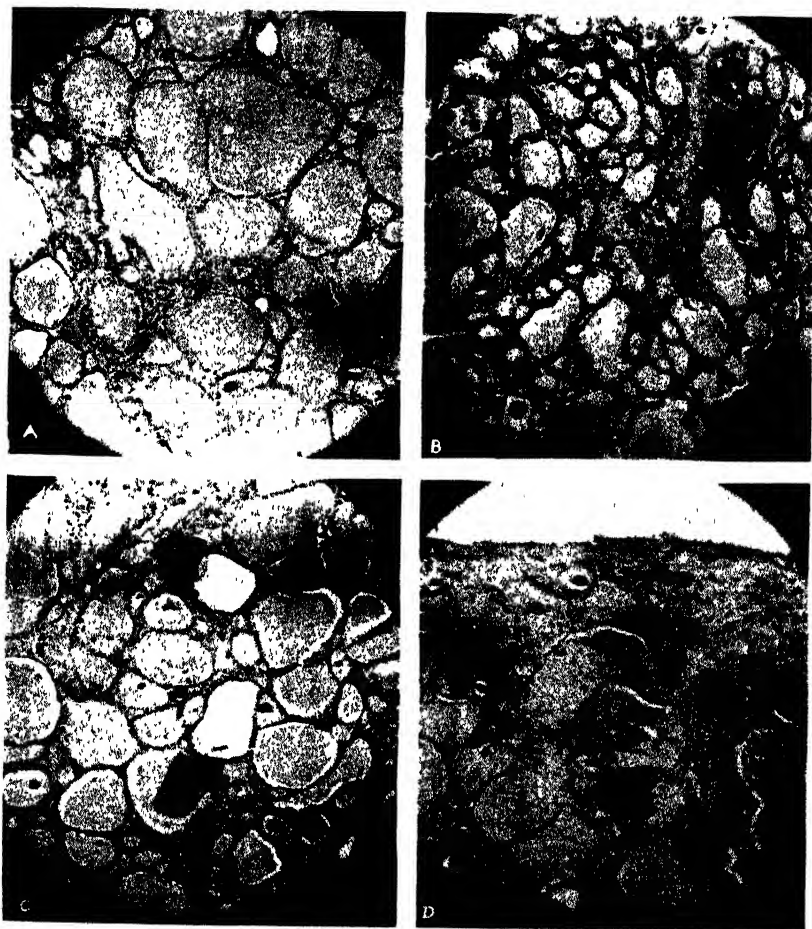


Plate III. Colloid tumor of human thyroid, fixed 24 hours in Zenker formol, washed for 24 hours in running water, adjacent tissue blocks were made. The tissue blocks were separated and passed thru the dehydrating reagent; a) fast dioxan; b) slow dioxan; c) iso-butyl alcohol; d) ethyl-alcohol-chloroform.

The greatest individual variation appeared in the Bouin fixed preparations of small intestine (Plate I). The fast dioxan method alone preserved the integrity of the villi and muscularis. Iso-butyl alcohol produced the greatest amount of distortion, the villi being shortened,

swollen, and the lamina propria and epithelium torn apart. Ethyl-alcohol-chloroform preparation showed short, thickened villi with shattering of the epithelium.

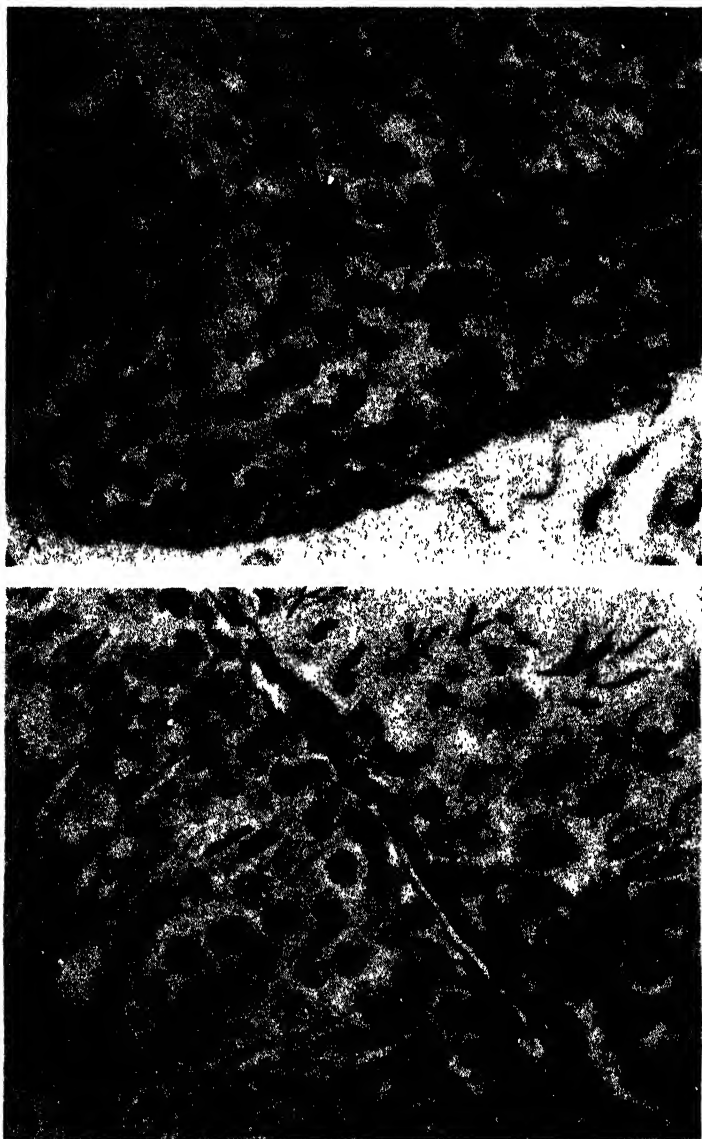


Plate IV. Testis of white rat fixed for 24 hours in Bouin's fixative hemisphered and placed directly into dioxan with several changes. After one month the tissue was removed from dioxan and one piece (a) was embedded with the usual dioxan method. The other piece of testis (b) was left in melted paraffin 9 days before embedding.

Formalin fixed muscle (Plate II) showed a definite shrinking of the muscle fibers in iso-butyl alcohol and a shattering in ethyl-alcohol-chloroform. The Zenker formol thyroid tumor showed similar changes, great shrinkage with iso-butyl alcohol and a shattering with ethyl-alcohol-chloroform (Plate III).

On the whole, the results of the experiment point to the greatest degree of tissue distortion in dehydration with ethyl-alcohol-chloroform, particularly following formal infixation (Chart 1). This suggests that while formalin may congeal or solidify the various cellular proteins, yet it does not render them unchangeable to subsequent treatment, and of the various dehydrating reagents dioxan produced the least amount of change. Zenker-formol fixed tissue appeared to be less subject to change during after treatment. The tissue however, did seem to be rendered less friable in dioxan than with other methods.

The fast dioxan method appeared to hold no particular advantage over the slow method, altho in the case of the formalin-fixed liver and the colloidal thyroid tumor some brittleness was produced by the fast method which was not true of the slow. As there is little time difference between the two methods, the author is inclined to favor the slower, or indirect method.

To determine the permissible variation of time, a rat testis² was fixed for 24 hours in Bouin's fixative, then hemisphered and placed directly into dioxan. The material was left in dioxan for one month with several changes, after which one piece was embedded in the usual manner and the other was left in *melted paraffin* at 60° C. for 9 days and then embedded. Both tissue blocks sectioned nicely with no tearing, shattering, or rasping sound. Microscopical examination revealed no shrinkage or distortion in the cellular elements (Plate IV).

Thus dioxan not only prepares tissues for easy sectioning, but produces no hardening effects before or during paraffin impregnation. It does not shrink the tissue and a wide time variation is permitted.

In addition to the experimental series, other material was treated with dioxan. The most remarkable demonstration of its non-hardening effect was the serial preparation of brains of the salamanders, *Cryptobranchus* and *Amblystoma*. The material was fixed in Cox-Golgi fluid for one month and became so friable that the greatest of care had to be exercised in the exchange of fluids. Following the slow dioxan method, the brains were serially sectioned at 10 microns without losing a section and with a minimum of fragmentation.

²The testis was selected for this particular test as it is particularly susceptible to shrinkage and distortion.

The author also experimented with the root tips of *Allium* and found less distortion with the slow dioxan method than with any other. It would be interesting to know if this method could be adapted to plant tissue in general.

CONCLUSIONS

1. Dioxan, regardless of the fixative, does not render tissue hard and brittle during the dehydrating process, or during paraffin impregnation.

2. The paraffin method is shortened by some 48 hours without sacrifice of or injury to the tissue.

3. Dioxan presents a wider time variation than hitherto experienced with any other dehydrating reagent.

4. Shrinkage and hardening of tissue, so often attributed to the hot paraffin, is directly related to the method of dehydration and preparation of tissue for the immersion in the melted paraffin rather than to the effect of the paraffin.

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HISTOLOGICAL APPLICATIONS OF TANNIC ACID AND FERRIC CHLORIDE¹

HENRY T. NORTON, *Department of Botany, University of California, Berkeley, California*

By using tannic acid and ferric chloride in conjunction with a cytoplasmic, cellulose-wall stain (light green or crystal violet) and with a nuclear, lignin stain (safranin), clear preparations of stems of various ages of *Pyrus* sp. (pear) and *Sambucus* sp. (elderberry) and of *Eichhornia* sp. (water hyacinth) root tips showing stages in the development of lateral roots, have been obtained. Such triple stained preparations surpass those stained by the usual combinations of safranin-light-green or safranin-crystal-violet in that the walls of cambial cells and meristematic cells of the growing points as well as young phloem and tracheary elements are more distinctly and permanently stained.

If, as in sections of *Pyrus* stems, the tissues concerned hold the safranin well in the subsequent dehydration, the delicate-walled cells may be stained with the tannic-acid-ferric-chloride combination during the process of dehydration following the staining with safranin. The following schedule has given good results with *Pyrus* sections fixed in formalin-acetic-acid-alcohol and cut at 12 μ :

(1) Transfer the slide from 50% alcohol to a 1% solution of safranin O in 50% alcohol. Stain 24 hours.

(2) Rinse slide for 10 seconds in 50% alcohol.

(3) Place slide in a 0.5% alcoholic solution of tannic acid (0.5 g. of tannic acid, C. P., Merck or Mallinckrodt, to 100 cc. of 50% alcohol) for 15 to 30 seconds.

(4) Pass the slide thru two Coplin jars of 70% alcohol leaving about 10 to 15 seconds in each.

(5) Place slide in a 1% solution of ferric chloride in 70% alcohol (1 g. of ferric chloride in 100 cc. of 70% alcohol) for 10 to 20 seconds.

(6) Continue the dehydration by leaving the slide in 80% alcohol for 10 seconds, in 95% alcohol for 20 seconds, and in 100% alcohol for 30 to 40 seconds.

(7) Transfer the slide from 100% alcohol to a 0.5% solution of crystal violet (gentian violet, Grubler, uncertified) in clove oil. Stain for 30 to 60 seconds.

(8) Rinse in xylol, transfer to clean xylol, and mount in balsam.

(9) Examine the finished preparation. The parenchyma and collenchyma cell walls should be stained purple, the walls of fibers red or purple, the lignified walls of the xylem red, the cambial cell

¹See Foster, A. S. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. *Stain Techn.*, 9, 91-2. 1934.

walls black, the nucleoli red, and the cytoplasm purple. If too much safranin has been washed out allow less time in the alcohols; if not enough has been washed out allow more time in the alcohols or add a drop of HCl to one of the jars of 70% alcohol.

If the safranin washes out easily, it is desirable to stain with the tannic-acid-ferric-chloride combination before staining with safranin. The following schedule has proven very satisfactory for cross sections of *Sambucus*, for cross and longitudinal sections of *Eichhornia* root tips, and for embryos of *Capsella*:

- (1) Transfer the slide from 70% alcohol to a 1% solution of tannic acid in 50% alcohol, and leave for 2 to 3 minutes.

- (2) Rinse for 15 to 20 seconds in each of two jars of 50% alcohol.

- (3) Place the slide in a 3% solution of ferric chloride in 50% alcohol for 30 to 60 seconds. The ferric chloride solution will blacken as the slides are passed thru it but this will not affect the staining. About 50 slides can be passed thru the alcohols and thru the ferric chloride.

- (4) Rinse the slide in 50% alcohol and then transfer to a 1% solution of safranin in 50% alcohol. Stain for 24 hours.

- (5) Rinse in 50% alcohol.

- (6) Partially destain in 70% alcohol but leave in more safranin than is desired in the finished preparation because the safranin will wash out in the other alcohols and will tend to become reduced in the light green.

- (7) Continue the dehydration in 80, 95, and 100% alcohols, leaving the slide in each about 10 to 30 seconds.

- (8) Transfer the slide from 100% alcohol to a 0.5 solution of light green in clove oil to which 7 cc. of 100% alcohol has been added for each 100 cc. of clove oil. Stain for 1 to 3 minutes.

- (9) Rinse the slide in 100% alcohol; transfer to xylol; and mount in balsam.

Sambucus stems stained according to the above method will have a pleasing appearance and all of the cell walls, including those of the cells of lenticels, will be distinct and clear. The cambial cell walls and parenchyma cell walls will be stained black, lignified walls will be red, and walls of the collenchyma cells will be a blackish red. The nuclei will be red, while the cytoplasm will stain bluish green.

Altho in the *Eichhornia* root sections the parenchyma as well as the meristem cell walls are stained black, the young lateral roots will stand out prominently because the cytoplasm of meristematic cells stains red whereas the cytoplasm of the parenchyma cells stains green.

Heartiest thanks are due to Dr. Adriance S. Foster for his constant coöperation and for the suggestion that the tannic acid method may be valuable in stem preparations.

NOTES ON TECHNIC

THE VIOLET AGAR REACTION AS A DIFFERENTIAL CHARACTERISTIC OF THE *Micrococcus catarrhalis* GROUP.—It was shown by Chapman and Berens¹ that the color of growths of staphylococci on crystal violet agar 1:300,000 could be used for differential purposes. When strains belonging to the *Micrococcus catarrhalis* group (gram negative, biscuit-shaped diplococci some of which were chromogenic) were plated on the violet agar medium, color changes similar to those observed with staphylococci were noted. Certain strains produced violet growths, others produced pale growths, while still others produced white growths.

The crystal violet agar used in these experiments had the following composition:—

Bacto-Beef Extract	3	g
Proteose Peptone, Difco	5	g.
Bacto-Lactose	10	g
Bacto-Agar	15	g
Crystal violet (National, cert) 1.0%	0.33	cc
Water to make	1000	cc
Final pH 6.8 ±		

In view of the implications attached to R and S differences and because of the marked dissociation which occurs in this group of organisms, the color of growths on violet agar was compared with the roughness or smoothness of the strains. The latter characteristics were determined by washing off growths from proteose lactose agar (the above medium minus crystal violet) into normal saline and, after allowing them to settle a few hours, classifying them according to the appearance of the suspensions. If the supernatant was clear, the culture was considered rough. If there was an opalescent or turbid supernatant suspension and a granular deposit, the culture was considered intermediate (SR). If the suspension was uniformly turbid, the strain was considered smooth.

Altho many strains were examined, only 34 were tested. The remaining cultures adhered so tenaciously to the proteose lactose agar that they could not be separated. Of the strains used for comparison, 6 produced violet growths, 15 produced pale growths and 13 were white. The correlation was as follows:—

¹Chapman, G. H. and Berens, C. Crystal violet agar as a differential medium for staphylococci. J. Bact., 29, 437. 1935.

²The strength was accurately calculated on the basis of dye substance and not on the crude weight of the dye.

Color of growth	Number of strains tested	Dissociants		
		Smooth	Intermediate (SR)	Rough
Violet	6	5	—	1
Pale	15	1	5	9
White	13	—	—	13

These results suggest that the violet agar reaction may be useful for the study of the *M. catarrhalis* GROUP.—GEORGE H. CHAPMAN, Clinical Research Laboratory, 604 Fifth Avenue, New York, N. Y.

A MEANS OF ELIMINATING WRINKLES IN SECTIONS PREPARED BY THE PARAFFIN METHOD.—Difficulty is often experienced in the preparation of paraffin sections, because of wrinkles in the paraffin and in the tissues. Such wrinkles are particularly common in large round or hollow structures, such as intestine, trachea, stomach, etc. In tissues of this sort the usual method of heating the paraffin strip on warm water often fails to remove the wrinkles and allow the material to flatten out on the slide.

By dissecting away the paraffin from the outside of the tissue, while the paraffin section is floating on the water on the slide, the wrinkles will be eliminated; and the section will flatten perfectly against the slide. This operation should be carried out prior to heating the water on the slide, for warm paraffin adheres to the dissecting needles. Furthermore, removal of the paraffin should be done under a low power dissecting microscope, so that the tissue will not be damaged.

Obviously, such a procedure involves delicate and tedious manipulation. For class use in biology courses, however, where only one or two sections need appear on each slide, the preparations are worth the effort expended. Even if serial sections are required, the method may be useful, for the results are perfect.—RUSSELL W. CUMLEY, Dept. of Zoology, Univ. of Texas, Austin, Tex.

LABORATORY HINTS FROM THE LITERATURE

A department devoted to abstracts of books and papers from other journals dealing with stains and microscopic technic in general

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

BOOKS

BECKER, ELERY R. and ROUNDABUSH, ROBERT L. Brief Directions in Histological Technique. 6 x 9 in., ix + 80pp. Paper. Collegiate Press, Ames, Iowa. 1935. \$1.00.

This booklet contains directions in general histological technic intended primarily for use in college classes in zoology and entomology. Special attention is given to the Zenker-paraffin-Delafield method to which a chapter of 20 pages is assigned. The following five chapters of the book deal with alternative technics in fixation, dehydration, embedding, staining, and mounting. A final chapter includes special methods as applied to protozoa, hydra, worms, earthworms, insects, blood, bone, embryos, and the demonstration of chondriosomes and Golgi bodies.

The book is bound in paper cover with spiral back, a method of binding which allows it very conveniently to lie flat on the laboratory table.—*H. J. Conn.*

CAMERON, GLADYS. Essentials of Tissue Culture Technique. Preface by Robert Chambers. Illustrations and chapter on Photomicrography by C. G. Grand. 6 x 9 in., xvi + 135pp. Cloth. 26 illustrations. Index. Bibliographies. Farrar and Rinehart, New York. 1935. \$3.00.

This compact volume of 135 pages, the first of its kind appearing in the United States, presents an excellent resume of the most approved methods of tissue culture. The author has gathered a wealth of experiences during her years of training at the Rockefeller Institute and in the laboratory of Dr. R. Chambers.

The text follows the idea expressed in the introductory paragraphs, namely, that "The technique of tissue culture has generally been considered somewhat forbidding, but its underlying principles are really simple and can be readily comprehended." Every step from the consideration given to the space and basic equipment, physiological solutions, embryo extract, etc., to manipulation of tissue culture and histological technic is presented in a clear and thoro manner. The bibliography of 64 titles comprises principally work published in English, altho a few important German and French references are also given.

The appendix on photomicrography by C. G. Grand is thoroly in keeping with the chapters on tissue culture. Mr. Grand fortunately has chosen simplified equipment. He gives ample yet concise directions that should insure successful photomicrographs from the hands of any microscopist who has mastered a few photographic principles.—*J. M. Thuringer.*

THE MICROSCOPE AND OTHER APPARATUS

CRAIG, R. and WILSON, C. A microtome knife holder for safety razor blades. Science, 81, 404-5. 1935.

Difficulties are commonly experienced in using razor blade holders commercially obtainable. A holder affording satisfactory rigidity and adjustment and proper bevel angle is fully described, which features curved surfaces of bottom and clamping blocks, eccentrically mounted holding pins and cooling arrangement.—*J. A. de Tomasi.*

KOPAC, M. J. Darkfield micromanipulation with an Ultrapaque illuminator. *Science*, **82**, 70. 1935.

For the study of the surface precipitation reaction in *Amoeba* the procedure used is as follows: Mount the organisms in hanging drop and place on the moist chamber. The needles of the micromanipulator are adjusted in the bright field of the substage lamp and condenser. The work and observations are carried on in the dark field supplied by the "Ultrapaque" illuminator (Leitz), consisting of a ring condenser on each objective. This permits arrangement of all degrees of illumination needed.—*J. A. de Tomasi*.

LENHARD, O. Zweinassstab-Mikrometer-Okular. *Zts. Wiss. Mikr.*, **51**, 68-9. 1934.

Within a micrometric ocular, 2 micrometric scales are brought to cross at right angles; one is fast and permits evaluation of distances from the end of an object, the other slides, allowing estimation of width or thickness at any given distance. It is thought of as a great help in following embryological developments.—*J. A. de Tomasi*.

NELSON, I. A. The use of razor blades for tissue sectioning. *J. Lab. & Clin. Med.*, **20**, 956-7. 1935.

A number of makeshifts are illustrated to show that various types of razor blades can be used for microtome work without special clamp holders. For a good support of the cutting edge, a discarded microtome knife or a similarly shaped piece of metal will do. The blade bevel is kept in close contact with the cutting edge of the old knife by means of a glue or cement. Duco Household Cement has proved satisfactory. The use of a couple of magnets is suggested for holding the blades together while the cement is setting.—*J. A. de Tomasi*.

SCHUCHTMAN, A. M. A simple device for the rapid observation of objects in lateral and ventral views. *Science*, **81**, 404. 1935.

A square observation chamber is built in a block of paraffin. The bottom and one of the side walls are supplied by two juxtaposed 90° prisms. Ventral or lateral views, or both, are obtained simultaneously by altering the position of the device with reference to the objective.—*J. A. de Tomasi*.

SCHMIDT, W. J. Ein Dehnungsapparat zum Gebrauch auf dem Mikroskop, hergestellt von E. Leitz in Wetzlar. *Zts. Wiss. Mikr.*, **51**, 54-65. 1934.

An apparatus is described for the study of stretching tissues, gels, etc., consisting of 2 parallel metal blocks between which the object lies. They are pulled apart in synchronous motion by a set of 2 cogwheels. Displacements are given on a calibrated drum, 1 revolution of which corresponds to 1 mm. total displacement of the 2 holding sides. This apparatus can be used on any common type of microscope stage, also immersed in liquids for work on imbibition.—*J. A. de Tomasi*.

SCHONTEN, S. L. Untersuchungen mit den Mikromanipulator. *Arch. exp. Zellforschung*, **17**, 429. 1935.

The author presents methods for isolated spores, bacteria, protozoa, etc., and describes growth changes in hanging drops. The technic described refers to his manipulator which he first devised in 1907 with improvements added principally in 1914 and subsequently.—*R. Chambers*.

SCOTT, J. A. An illuminator for the binocular dissecting microscope. *Science*, **81**, 595-6. 1935.

Especially in the examination of nematode larvae within water drops, it has been noticed that illumination in the orthodox way is not equally distributed to both eyes. This device is designed to correct such condition. Two bulbs, whose distance apart can be regulated, send their respective light beams thru two 500 cc. flasks of alcoholic CuSO_4 soln. These may be adjusted so as to give the two beams a correct angle at the microscope mirror. The light then passes a ground glass and a window of plain glass. In assembling the outfit in a light-tight box, the flat side of the bulbs' filament should face the flasks.—*J. A. de Tomasi*.

MICROTECHNIC IN GENERAL

CARLSON, J. G. A rapid method for removing cover glasses of microscope slides. *Science*, **81**, 365. 1935.

A few hours immersion in a mixture of 90 parts xylene and 10 parts *n*-butyl alcohol will loosen up any cover slip. Anilin dyes, however, will also be taken off.—*J. A. de Tomasi*.

CROSSMON, G. A paraffin block cooler for use with the microtome. *Science*, **81**, 466-7. 1935.

In order to obtain paraffin ribbons that show little or no compression, a stream of cold air is directed at the block on the microtome. This is accomplished by passing compressed air thru a CaCl₂ tube and a copper coil enclosed in a small cooling chamber. This is filled with ice or a freezing mixture. The cold air outlet is kept at a distance proportionate to the amount of cooling wanted. A few sections are cut previous to chilling so as to establish a lead for the ribbon. The air draft does not disturb and no electrification has been noticed.—*J. A. de Tomasi*.

FAY, A. C. A generally applicable method for the enumeration of microscopic objects. *J. Lab. & Clin. Med.*, **20**, 1088-9. 1935.

Method is as follows: Place 0.1 ml. or 0.1 g. of the specimen on a 3 x 1 in. chemically clean glass slide. If necessary a few drops of water or other liquid may be added for spreading. Any objective can be used, provided the proper factor is used in the final evaluation. Factors are given in 2 tables. With aid of a stage micrometer, record the tube length which will give diameters of 1.57, 0.351, and 0.157 mm. for the fields of the low powers, high dry, and oil immersion objectives. The areas represent 1/1,000, 1/20,000 and 1/100,000 of the area of the slide, respectively. If 0.1 ml. of the specimen is employed, the average number of objects per field multiplied by 10,000, 200,000, or 1,000,000 will give the count per ml. Other basic measurements and factors are given for counts based on continuous strip readings.—*J. A. de Tomasi*.

JOHANSEN, D. A. Dehydration and infiltration. *Science*, **82**, 253-4. 1935.

Successful dehydration and infiltration are not dependent upon successful fixation: the two processes are mutually exclusive. It is assumed that good infiltration occurs when the water is replaced without the water-absorbing capacity of the tissue being destroyed. A dehydrating medium, therefore, should mix in all proportions with water, ethyl alc., paraffin and balsam. These requirements eliminate practically all fluids in common use. Normal butyl alc. is also one of them since with many plant tissues it has been proven to carry about desiccation. Dioxane and tertiary butyl alc. prove to be satisfactory; they give perfect preservation of the image, remove free water but leave the water-absorbing capacity unimpaired, produce no hardening, and do not affect staining properties.—*J. A. de Tomasi*.

NELSON, I. A. The use of proteins for embedding small tissues for sectioning by the freezing method. *J. Lab. & Clin. Med.*, **20**, 964-8. 1935.

This is not an impregnating method and applies chiefly to tissues which hold together within themselves. It is based upon the use of permeable cellophane shaped into an embedding box and acting as a semi-permeable dialyzer. Blocks are formed in such a box by the coagulation of blood or egg proteins. Oxalated whole blood left over from clinical tests and containing at least 14 g. of hemoglobin per 100 cc. may be used. It is emulsified by addition of a small bit of saponin powder. When the bloods from various groups are pooled, it is possibly better to take the cells of each blood before adding them to the pool. Separate and combine all the blood plasmas from their cells, take and pool these and then mix equal parts of this lake with the combined plasma. Whole raw eggs may be used instead of blood and merely require stirring of the yolk into the albumin. The procedure of blocking is as follows: Place the cellophane box with the tissue particles in a small dish. Pour the emulsoid protein into the box. Stir and orient the pieces. Pour into the dish enough 20-40% formalin, neutral or slightly alkaline, to fill it up to a little below the level of the emulsoid within the box and let it stand for 2-4 hr. Peel off the cellophane, rinse the block in running water and cut it on the freezing microtome.—*J. A. de Tomasi*.

STEWART, J. D. and MENNE, F. R. A method of staining amyloid in permanent gross specimens. *J. Tech. Meth. & Bull. Int. Assoc. Med. Museums*, 13, 60-1. 1934.

Spleen portions preserved in 2% Lugol's soln. and 2% H_2SO_4 give the best results: the amyloid stains mahogany-brown, the surrounding tissues light yellow. Use at least 10 times as much fluid as the volume of the tissue. The tissue should be well fixed in formalin to prevent turbidity in the preserving liquid.—*J. A. de Tomasi*.

WILSON, C. and HOCKADAY, J. S. An easy way to reduce electrification of paraffin ribbons. *Science*, 82, 306. 1935.

Static electricity generated at the knife edge can be eliminated from paraffin sections by allowing leakage of the charge into the air. Boiling water present in a sectioning room will furnish sufficiently high humidity to permit cutting ribbons as thin as 2μ in any weather.—*J. A. de Tomasi*.

WYCKOFF, R. W. G. Ultraviolet microscopy as a means of studying cell structure. *Cold Spring H. Symp. Quant. Biol.*, 2, 39-44. 1934.

Ultraviolet microscopy provides a tool capable of solving many problems in cytology. The author discusses the rationale of the technic, with its advantages and limitations. With ultraviolet light one can see things having about half the diameter of the smallest object discernible with visible light: structures in living cells that are invisible in ordinary light may also be seen.—*M. W. Jennison*.

ANIMAL MICROTECHNIC

ARNIM, S. S. A method for preparation of serial sections of teeth and surrounding structures of the rat. *Anat. Record*, 62, 321-30. 1935.

Material, white rat. Best fixation, formalin-mercuric chloride acetic acid. Store 5-7 days in 10% formalin, wash in running water, dehydrate in abs. alc. and stir continuously in a desiccator. Embed in celloidin. Run blocks down to water and decalcify by means of a 7% aq. soln. of acetic acid until test of acid soln. with ammonium oxalate gives no ppt. Then dehydrate the blocks with propyl alc. and re-embed, starting with 3% celloidin. Harden, clear and embed in 42° C. paraffin. Cut on sliding microtome 7μ thick. Mount by special technic using propyl alcohol, and stain with dilute Delafield's hematoxylin and aqu. eosin. Method shows retained debris in sulci and on surfaces of teeth, the enamel lamellae, and outline of enamel rods in serial sections $3-10\mu$.—*S. I. Kornhauser*.

BALLOWITZ, E. Zur Fixierung und Konservierung der Spermiozeugnen. *Zts. Wiss. Mikr.*, 51, 51-3. 1934.

The male ducti deferentes and the female receptacula seminis from freshly killed insects are placed in a 0.75% NaCl soln. The "Spermiozeugmen" are isolated by teasing on a glass slide, and handled by the following technic: Remove the tissue debris and turn the preparation as a hanging drop over a 1-2% osmic acid soln. for 15 min. Stain lightly with eosin and mount in glycerin, or spread the fixed material on a cover slip; air dry and fix to the glass by passing several times thru an alc. flame. Stain such cover slip preparations by letting them swim on top of a dye solution. Rinse quickly in dist. water, air dry and mount in balsam.—*J. A. de Tomasi*.

BAUER, K. Über pathologische Reaktionen im embryonalen Organismus nach Einwirkung chemischer und physikalischer Mittel. *Virchow's Arch.*, 294, 477. 1935.

Material: Chick blastoderms. Fixation: Zenker's fluid or Held's 'green fluid' (chromic acid, formalin and acetic acid). Embedding: celloidin-paraffin. Sections treated by modification of Held's method for staining with molybdenum-hematoxylin. Method as follows: Iron alum (40° C.), 20 min. Dilute molybdenum-hematoxylin (at least 4 months old) at 50° C., several hr. Wash in dist. water. Differentiate in ferrocyanpotassiumborax and wash with dist. water for 1-10 min. Place in 2% uranyl acetate, 30 min. Rinse in dist. water. Graded alc. Treat 5 min. in the following soln: Carboxylol, abs. alc., and acetone, to which has been added several drops of alcoholic erythrosin. Results: cytoplasm, blue; intracellular and intercellular substances, red.

An additional method for uranhematoxylin is given: Composition: hematoxylin, 1 part; 70% alc., 200 parts; uranyl nitrate, 5 parts. Place sections in this soln. for several hr. or days at room temp. Wash and differentiate in 2% alum. Wash in dist. water 5-10 min. and differentiate in 2% uranyl acetate for 30 min. Alcohols, xylol, balsam. Results: cytoplasm, blue; intercellular substances blue (depth depending upon treatment with alum).—*H. E. Jordan.*

CASATI, A. Ricerche sperimentali intorno all'azione dei Raggi X sul midollo osseo. *Radiobiologia*, 1, 91-5. 1935.

Two groups of rabbits are X-rayed twice at 15-day intervals by irradiations of 8 H or 400 r. using 140 KW. and 2.5 mA at a distance of 40 cm. with a 0.5 mm. Zn and 2 mm. Al filter. The first group, every other day after irradiation, is given 8 injections (3 cc. each) of trypan blue in 1% NaCl; the second is injected, then irradiated. After 30 days, tissue pieces are fixed in formalin, embedded in paraffin, cut and stained with eosin. In both groups the bone marrow "transitional" cells show the action of irradiation. These forms, however, do not take the vital stain, are considered derived from myoblasts, and not a part of the reticulo-histocytic system. Reticular cells are normal, some of them presenting trypan blue granules deposited on the fibrils.—*J. A. de Tomasi.*

CHAMBERS, R. Disposal of dyes by proximal tubule cells of chick mesonephros in tissue culture. *Proc. Soc. Exp. Biol. & Med.*, 32, 1199-1200. 1935.

Aqueous solutions of dyes in various concentrations were mixed with the usual tissue culture medium in which were planted fragments of functioning mesonephros of a 9-11-day chick. Isolated segments of the tubules regenerate their cut ends and become converted into closed sacs into which progressive accumulation of color can be observed. Dyes used were: neutral red, C. I. No. 825; Nile blue sulfate, C. I. No. 913; xylene cyanol FF, C. I. No. 715; amaranth, C. I. No. 184; acid fuchsin, C. I. No. 692; orange G, C. I. No. 27. Dyes appear to be taken up by proximal tubules either by passive infiltration of a lipid soluble dye regardless of temperature, within viable limits, or by an infiltration dependent on the metabolic activity of the cells. Sulfonated dyes actively taken up by renal cells could be arranged in series, at one end of which were dyes which passed into the lumina of the tubules directly, and at the other end of which were dyes collected in segregation vacuoles in the cells with little tendency to pass into the lumina of the tubule.—*M. S. Marshall.*

DAVID, L. T. A method of preparing paraffin sections of bone. *Science*, 82, 179 1935

Pieces of bone as large as 2 cm. × 2.5 cm. × 2 mm. can be used. Decalcify with 5 to 10% HNO₃, followed by 5% aq. Na₂SO₄ sol. (24 hr.) and running water (24 hr.). Dehydrate, clear in 1:1 abs. alc. and chloroform, 2 changes of chloroform; pass thru several changes of paraffin (M. P. 47° to 40°), embed in harder paraffin (M. P. 56° to 58°). Expose the side to be sectioned to water until the tissue shows definite swelling (2-10 days); dry the surface and dip in melted paraffin. Flood clean slides with the following fixative: Water 100 cc., standard water-glass sol. 1 cc., concd. NH₄OH, 1 cc. Add sections, warm slightly, drain excess fluid. Dry slides at least 48 hrs. Good results have been obtained with small bones of various structure and hardness; the staining calls for hemalum and eosin.—*J. A. de Tomasi.*

FAY, A. C. The detection of formaldehyde in milk by means of the methylene blue reduction test. *J. Dairy Sci.*, 18, 327-31. 1935.

Confusion has frequently arisen from analyses of milk samples which showed a short reduction period (less than 30 min.) and at the same time a low plate count. Since in such cases tests for formaldehyde always proved positive, the methylene blue procedure might help in locating cases of adulteration with this preservative. Serial concentrations of HCHO from 1:100 to 1:100,000 were made up in milk which was plated out immediately, and after 24 hr. at 21° C. Duplicate controls and also methylene blue reduction tests were set up. Milk alone reduced the dye in 660 min.; a concentration of 1:100,000 of the preservative cut the time down to 259 min.; 1:1000 to 21 min. Higher concentrations increasingly inhibited reduction. The range of dilutions between 1:15,000 to 1:25,000 established close limits; in greater concentrations the preservative is readily tasted; at higher dilutions it is ineffective.—*J. A. de Tomasi.*

GERSONI, C. S. A window technique for fetal observation. *Anat. Record*, **63**, 169-73. 1935.

Altho the present paper deals only with gross examination, it is of interest to microscopists because window technic is often adapted to the study at close range of histological details of the development of blood vessels, lymphatics, and nerves, and of the action of vital stains in the body.

White rats pregnant two weeks are depilated on the abdominal wall, incised, and a portion of the wall removed. Silk loops are placed anterior and posterior to a single foetus. These loops are drawn thru holes in the anterior and posterior borders of a window consisting of celluloid with a tin frame. The foetus in the uterus is drawn against the inside of the window which is sewed to the belly wall. Collodion makes the joint air tight. The movements of the foetus may now be observed until term is reached. Cutting the loops about the uterus allows the observed foetus to be born with its litter mates.—S. I. Kornhauser.

GRADWOHL, R. B. II. A new method of staining blood platelets. *J. Amer. Med. Assoc.*, **105**, 1031. 1935.

The following technic is a modification of the method described by Fonio.

Reagents used: Filtered 14% $MgSO_4$ soln. Methyl alc. (National Aniline and Chemical Co.) for counting blood platelets. Giemsa stain. Neutral dist. water, tested for neutrality as follows: Add 5 cc. of the dist. water to be tested to a few hematoxylin crystals placed in a chemically clean test tube. If water turns pink in 2-5 minutes, it is neutral; if it becomes pink before 1-2 minutes, it is alkaline; if it remains yellow for 5 minutes or longer, it is acid. (To neutralize acid water, use 1% Na or KOH; for alkaline water 1% HCl or acetic acid. Add reagent to water a drop at a time and test with hematoxylin crystals.)

Technic: After producing hyperemia by immersing patient's hand in hot water and drying with sterile gauze, place 1 drop of $MgSO_4$ soln. on the end of the finger and puncture thru the drop. When there is a mixture of about 1 part blood and 9 parts $MgSO_4$, mix with needle point and transfer a drop of mixture to a fat-free slide. Smear with a cover slip as for a blood film. Fix in methyl alc. for 5 min. in a Coplin jar. Wash in neutral dist. water to remove any $MgSO_4$ sol.

Mix 5 cc. of Giemsa stain and 5 cc. of neutral dist. water by shaking slightly between each drop. Flood slide with stain for 30 min. Wash in neutral dist. water.

Method of counting: Count all erythrocytes and blood platelets in consecutive fields until 250 erythrocytes have been counted. Calculate the number of blood platelets in 1 cu. mm. of blood by the following formula: Patient's red count \times number of blood platelets counted $\times 1/1000$ = number of blood platelets in 1 cu. mm. of blood. This method eliminates precipitate common in Fonio's method.—Elizabeth F. Genung.

KAISERLING, H. Das Gewebsbild des fieberhaften Rheumatismus. XVII. Mit. Veränderungen der feineren Muskelnerven beim Rheumatismus. *Virchow's Arch.*, **294**, 414. 1935.

In the course of preparing sections by the usual silver impregnation method a solution of dextrose (strength not given) was used in place of formalin as a reducing agent.—H. E. Jordan.

KIMURA, R. and MURAKAMI, J. Lymph als Nährmedium zur Zuchtung der Gewebe. *Arch. exp. Zellforschung*, **17**, 335. 1935.

Lymph is obtained from rabbit of about 3,000 g. weight thru the vas efferens of the popliteal lymph node after having ligated the vas till it swells. One may also obtain lymph from the vasa afferentia which lie under the gland. Within half an hour one is able to obtain 1 cc. lymph. Massage of the peripheral parts of the lower extremities assists the operation.

The lymph coagulates soon after adding tissue extract. No heparin is necessary. Spleen cultures grow much better than in blood plasma.—R. Chambers.

LANDGRAF, G. Beitrag zur Markscheidenfärbung. *Zentbl. allg. Path.*, **63**, 56. 1935.

Application is made of chlorophyll as a myelin sheath stain with a red nuclear counterstain. Dissolve Chlorophyll, spissum (Bayer-Meister-Lucius) 2 g. in 50 cc. of a mixture of 70% alc. and acetone equal parts by heating slowly just to boiling. Filter

after cooling. Use formalin-fixed frozen sections in water; rinse briefly in 75% alc.; chlorophyl soln. 10 min.; rinse in 75% alc.; several min. in dist. water; 2-3 min. in Kernechtrot (100 cc. 5% $\text{Al}(\text{SO}_4)_3$ solution + 0.1 g. Kernechtrot) as counterstain; wash in water; embed in levulose syrup. Myelin sheaths stain dark green, ganglion cells red.—*H. A. Pavenport.*

LEWIS, M. R. Effect of the vital dye fluorescent X (reduced neutral red, Clark) on living chick embryo cells in tissue cultures. *Arch. exp. Zellforschung*, 17, 96-105. 1935.

Technic: Neutral red was reduced by sodium dithionite; buffered heavily at about pH 4 to 6; separated crystals were washed many times with water; 1 sample was reduced in the presence of a phosphate buffer, the other in the presence of an acetate buffer; recrystallized from methanol and water. Staining reactions of fluorescent X on living tissues usually were made 1:8000 parts soln. of dye in culture media.

Cultures of chicken embryo tissue were prepared in hanging drops of Locke-Lewis soln.; grown at 39° C. for 2-3 days; culture containing dividing cell selected and a cell in mitosis microscopically located; cover glass removed; under surface of growth bathed with a few drops of sterile medium. Cultures were fixed at varying periods by means of Zenker-formol, containing 0.5% acetic acid and later stained with Harris' hematoxylin.

Results: Fluorescent X was taken up by living cells in tissue cultures and accumulated in certain granules as the reduced (yellow) dye. After 24-48 hr. the dye in the stained bodies became oxidized to neutral red, remained red until the cell degenerated, then returned to the reduced (yellow) form.

Abnormal mitosis occurred in the presence of the fluorescent X in the reduced state; abnormalities became fewer as the dye changed from yellow to red; within 48 hr. almost all divisions were again normal.—*Robert Chambers.*

LUDFORD, R. J. The cytological action of methylene blue. *Arch. exp. Zellforschung*, 17, 339-59. 1935.

Dyes: "Tabloid methylene blue" of Burroughs Wellcome, Gurr's "methylene blue for injection," and "standard stain" methylene blue of British Drug Houses, Ltd.

Tissue: Cover glass cultures of (a) chick embryo heart in fowl plasma, and in fowl serum, (b) rat embryo heart in rat plasma, and in rat serum, (c) mouse embryo heart in mouse serum, and in rat plasma.

The dye was used in concn. of 1 part in 10,000 parts of Ringer soln. It was applied in each case by raising the cover glass of the culture and filling the well of the hollow glass slide. The culture was resealed and returned to the incubator for 4-8 min. It was then mounted in a drop of the same Ringer methylene blue soln. alone on a glass slide and examined at room temp.

Results: Methylene blue stains the mitochondria, and cytoplasmic vacuoles and granules of living cells. Mitochondrial staining is inhibited by bright light, low temp. and by cyanide. The dye readily penetrates the nucleus and stains the nucleolus followed by coagulation. Dead cells stain diffusely. Both normal and malignant cells react alike to the dye, Janus green, toluidine blue and brilliant cresyl blue act similarly.—*Robert Chambers.*

MANNA, LA. Über Myoblastome. *Virchow's Arch.*, 294, 663. 1935.

Object: To demonstrate cross striations in tumors of myoblast origin following fixation with Jores' fluid and silver impregnation according to Achucarro (modified by del Rio Hortega). Method: paraffin embedding, thin sections; silver impregnation; dist. water, then stain as follows. Concentrated FeCl_3 , 20 min.; dist. water, wash until yellow color is gone; stain for 20 min. in soln. made up as follows: 10% iron hematoxylin dissolved in abs. alc. is added in ratio of 1% to dist. water and to 3 cc. of this dilute soln. is added 1 drop of 10% aq. FeCl_3 . Slides are held horizontally and covered with the stain. Wash with water. Differentiate with following soln: 100 cc. of 10% FeCl_3 plus 2 cc. 10% iron hematoxylin. Control differentiation with microscope. Wash with dist. water with slides in vertical position. If cellular elements are over-stained, decolorize by allowing a satd. aq. soln. of Li_2CO_3 to flow over sections. Rinse in dist. water, pass thru graded alcohols and xylol and embed in balsam. Results: Cross-striations, nuclear structure, intra- and intercellular bile capillaries clearly demonstrated.—*H. E. Jordan.*

MANSTEIN, B. Über die Einwirkung von sauren und alkalischen Lösungen auf Organ-schnitte und ihre Beziehungen zur Karyolyse. *Virchow's Arch.*, **294**, 120. 1934.

To demonstrate the effect of acid or alkaline solutions on nuclei of different tissues, thin frozen sections cut under aseptic conditions are kept at either 4° C., 20° C. and 37° C. or exposed to buffer solutions of KH_2PO_4 and K_2HPO_4 (pH 5.0, 6.0, 7.0 or 8.0) for 24-48 hr. and stained with Mayer's hemalum.—*H. E. Jordan.*

OKAJIMA, K. Färbung durch Eisenchloridalizarinlack (Omnichrom). *Folia Anat. Jap.*, **13**, 383-4. 1935.

This stain, named "Omnichrom" by the author, is particularly praised for its simplicity of preparation and use. The stain consists of 2% aq. FeCl_3 , 10 cc.; sat. aq. alizarin red S, 60 cc. Before use dilute with twice as much water. The paraffin, celloidin or other sections pass from water into the stain for 0.5-2 min. Wash in tap water, dehydrate in alc. with care not to overdo. Results: chromatin, collagenous tissue, cell wall and membrane, fibrin, cytoplasm of nerve cells, acidophile and basophile cells stain violet or dark violet; ground mesh of young bone, violet-red; erythrocytes, nuclear bodies, keratin and the lense fibers, orange-red.—*J. A. de Tomasi.*

OKINO, K. Metallimpragnationsversuche an den Erythrocyten. II. Imprägnierung der polychromatischen Erythrocyten bei der experimentellen Phenylhydrazinämie. *Folia Anat. Jap.*, **13**, 183-8. 1935.

It was previously proved that with normal erythrocytes, the stronger their negative charge, the more silver they take on by impregnation. Polychrome erythrocytes carry a stronger negative charge than orthochrome ones. Will they then also show high affinity for Ag-ions? Rabbits made anemic by phenylhydrazine are the source of polychrome erythrocytes. The air dried blood smears are treated with the usual silver reagent (Okino, K., *Folia Anat. Jap.*, **12**, 373-81. 1934) with a further addition of 25 drops of 2% AgNO_3 to 100 cc. of the reducing soln. Giemsa stains serve as controls. Results: Stronger polychrome cells, packed full of very fine dark brown particles, stain uniformly dark brown; light polychrome cells either show brown-yellow particles or scattered precipitates on a colorless background. Orthochrome erythrocytes instead appear quite clear and colorless.—*J. A. de Tomasi.*

PAILOT, A. Contribution à l'étude des maladies intestinales du ver à soie, deux types nouveaux de dysenterie non infectieuse. *Ann. Inst. Pasteur*, **54**, 627-48. 1935.

In the study of two new types of intestinal disturbances in the silkworm, the technic employed was as follows: Fix either in saline formol (40% formalin, 20 parts; saline, 80 parts) or in Regaud's bichromate-formol (40% formalin, 20 parts; 3% $\text{K}_2\text{Cr}_2\text{O}_7$, 80 parts). After about three weeks of chromate fixation, embed in paraffin. Stain, according to Kull's method, in warm 20% acid fuchsin in anilin, followed by 0.5% toluidine blue. Differentiate in 0.5% aurantia. Chondriome and nucleoli appear red; chromatin, blue. (No further details of technic given in the paper)—*J. A. de Tomasi.*

RATHMELL, T. K. and JONES, H. W. The preservation of blood films. *J. Lab. & Clin. Med.*, **20**, 954-6. 1935.

The method is intended to be an improvement on the usual Canada balsam technic. The stained dry blood film is covered with a thin coating of lacquer (lacquer No. 14. J. H. Weil Co., 1315 Cherry St., Philadelphia) by means of a No. 16 artist's sable brush. The preparations will be dry in 60-75 sec. and remain impervious to attack by xylol or cedar oil. Under a dry objective the film has an appearance superior to that of the unmounted slide. Under oil immersion, results are equal to such as obtained with conventional mounts. Use a dry slide and apply smoothly at one stroke.—*J. A. de Tomasi.*

SAGENIEHL, H. Über die Blutversorgung der alternden Herzen. *Virchow's Arch.*, **294**, 147. 1934.

Object: To keep lumens of coronary arteries open for histologic study of character of wall and size of lumen. Healthy hearts removed from body and cleaned, kept for 24 hr. on ice covered with gauze; then placed in incubator at 39° C. for 6 hr. Canula inserted in coronary artery and Ringer's soln. (at 39° C.) injected at pressure of 180 mm. Hg, followed by barium-gelatin (at 39° C.). Heart is then plunged into ice-cold forma-

lin. After fixation for 48 hr. small pieces of heart wall are removed and decalcified in formic acid plus equal parts of 10% formalin (decalcification because hearts were from old people). Pieces are embedded in paraffin, sectioned and stained with Weigert's, hematoxylin-Sudan, or Kernechtrot.—*H. E. Jordan.*

WINKLER, J. E. Über die Beziehungen Zwischen Plasmazellen und Mikrogliazellen in dem nervösen Parenchym der Schlafkranken. *Zts. gesam. Neurol. u. Psychiatrie.*, **153**, 160 4. 1935.

Coincident staining of microglia and plasma cells is described. A) Microglia. Brain formalin fixed, frozen sections, wash 3 times in hot water, then into 0.3% alcoholic soln. *Mastix* for 2-8 hr. Wash 3 times in dist. water; place in 2% HBr 14-24 hr. Wash 3 times in water; place in 2.5% Na_2CO_3 6-24 hr.; impregnate in Hortege's ammoniated silver soln (diluted 5-8 times) for 0.5-2 min. Rinse quickly and reduce in 20% unneutralized formalin. B) Plasmacells. Transfer sections stained as above to May-Grunwalds stain diluted 6 drops to 60 cc. dist. water, 24 hr. Wash thoroly in dist. water, differentiate in abs. alc. until sections appear pale blue. Creosote-xylene, xylene, balsam. Plasma cells deep blue, microglia brown.—*H. A. Davenport.*

YASUZUMI, G. Über den isoelektrischen Punkt der tierischen Gewebe. IV. Mitteilung. Über den Einfluss von Fixierungsmitteln auf die Farbbarkeit der Erythrozyten. *Folia Anat. Jap.*, **13**, 333-42. 1935.

The influence of fixatives on the stainability of erythrocytes is studied on mouse blood cells. Their isoelectric point is determined colorimetrically by means of the Rovibond tintometer. The influence of H-ion concn. is studied by holding the cells 2-30 min. in isotonic salt soln. at various reactions, followed by alcohol fixation. The pH of 17 different fixatives is determined colorimetrically as well as potentiometrically, whenever possible. The fixatives' capacity to coagulate hemaglobin is tested on the filtrate obtained from the pptn. of the blood stroma by means of the phosphate-citric acid buffer soln.

When erythrocytes treated with salt soln. at various reactions and alcohol-fixed are stained with toluidin blue and also with Ponceau, the color intensity decreases toward the acid range with the basic dye and toward the alkaline with the acid dye. Trichloroacetic acid, $\text{K}_2\text{Cr}_2\text{O}_7$, AgCl , CrO_3 , and Muller's and Zenker's fluids, tend to displace the isoelectric point in the direction of alkalinity; picric acid H_2PtCl_6 , OsO_4 , Helly's, Orth's, Rabi's and all mixed fluids containing OsO_4 , shift it toward the acid side.—*J. A. de Tomasi.*

PLANT MICROTECHNIC

BECKER, W. A. and SKUPIENSKI, F. X. Observations protoplasmiques vitales sur *Basidiobolus ranarum* Eidam. *Compt. Rend. Acad. Sci.*, **200**, 1620-2. 1935.

The fungus *Basidiobolus* was grown on 4% beer wort agar or directly in various concentrations of beer wort. Neutral red, methylene blue and cresyl blue were added to the solid or liquid cultures in concentrations of 1:5,000 or 1:10,000. The growth was normal while the hyphae remained unstained. This is attributed to a decrease in pH of the medium which fell from neutrality to 5.24 in 3 months. Similar results (using neutral red only) were secured by microcultures under a cover slip resting on 4 drops of wax. At higher concentrations of the dye, some structures at first take on the stain, especially the vacuoles, cytoplasmic granulations and sometimes their membranes, but soon the color gradually disappears in definite shapes and the cell resumes further growth activities; the cytoplasm and nucleus migrate to the upper end and develop a transverse wall framing off the rest of the empty mycelium. At times even the nucleolus will temporarily take on the dye.—*J. A. de Tomasi.*

FREY-WYSSLING, A. Über die Verschiebungsfiguren zellulöser Zellwände. *Zts. Wiss. Mikr.*, **51**, 29-36. 1935.

The origin of displacement patterns in plant cell walls is dependent upon several conditions: membranes must be of pure cellulose and the object must display an ideal fibrous structure, i. e., the orientation of the rows of micellae must be parallel to one another and to the morphological axis of the cell. Displacement patterns may occur not only with bast fibers but also with membranes of other cells like the hourglass cells of leguminous seed shells. These patterns are not to be taken as lattice figures in strict crystallographic sense, but rather as "strains" in their micellar structure.—*J. A. de Tomasi.*

LENOIR, M. Étude des deux variétés de chromatine dans le noyau du *Lilium Martagon*. Compt. Rend. Soc. Biol., 118, 1554-6. 1935.

Ovaries of *Lilium Martagon* offer good material for the study of two nuclear chromatin, the reticulon and the nucleolin. Fix in Bouin-Duboscq-Brasil (Compt. Rend. Soc. Biol., 103, 1258, 1930) and stain with the basic fuchsin, malachite green double stain as previously outlined (Compt. Rend. Soc. Biol., 104, 1282, 1930). The reticular chromatin takes on the fuchsin; the nucleolar, the malachite green. After several years, however, the stain fades, particularly the green.—*J. A. de Tomasi*.

SCHMELZER, W. Bemerkungen zur Anwendung saurer Karminlösungen auf pflanzliche Gewebe. Zts. Wiss. Mikr., 51, 66-7. 1934.

It is found advantageous to increase the acidity of borax or lithium carmin solutions when intense and durable plant tissue stains are wanted. Add, for example, 1 drop of concn. HCl to 5 cc. of a mixture in equal parts of 70% alc., the carmin soln., and glycerin.—*J. A. de Tomasi*.

SCHMIDT, W. J. Über eigentümliche Veränderungen von Zellulosefaden in Kanadabalsampräparaten. Zts. Wiss. Mikr., 51, 2-11. 1935

After several years of inclusion in balsam, cellulose threads from epidermic cells of *Cobaea scandens* seeds undergo considerable changes near the edge of the cover glass. At first some pseudomorphic modifications take place which do not mar the shape of the threads. Later a thinning sets in, accompanied by development of particles and rods of pseudocrystalline nature with a general optical orientation common to the rest of the thread. The positive double refraction of threads shifts gradually to a negative double refraction. It is considered that the balsam as well as O₂ from the air are responsible for these chemical changes comparable to cellulose nitration and acetylation. It is possible, moreover that acid products developing from the neutral balsam may bring about the reaction.—*J. A. de Tomasi*.

MICROÖRGANISMS

CHAPMAN, G. H. and BERENS, C. Crystal violet agar as a differential medium for staphylococci. J. Bact., 29, 437-48. 1935.

Among other chemical agents, crystal violet (National Aniline, certified) and basic fuchsin (National Aniline, certified) are selected for comparative tests. Dilutions ranging from 1:100 to 1:1,562,000 are measured into sterile tubes in 0.5 cc. quantities. Two strains of staphylococci, one pathogenic and positive to hemolysis and coagulase tests, the other negative, show best differentiation with crystal violet. Proteose lactose agar added with crystal violet in an optimum ratio of 1:300,000 is used for plating. Of the 594 strains tested 51 grow orange, 193 violet colonies and react positively to hemolysis and coagulase tests, while 350 are white and do not correlate. The effect of crystal violet solutions on the color of suspensions of staphylococci is also studied.—*J. A. de Tomasi*.

COWDRY, E. V. and HEIMBURGER, L. F. Morphology of bacilli of rat leprosy. Proc. Soc. Exp. Biol. & Med., 32, 1422-3. 1935.

It is suggested that the freezing and drying method of fixation of slides preparatory to staining acid-fast organisms in tissues with the Ziehl-Neelsen technic (Gersh, et al.) may contain some unsuspected flaw, or that the bacillus of rat leprosy and the human tubercle bacillus in tissues are less granular than commonly supposed. The usual fixation in 10% formalin in abs. alc. or in Regaud's fluid produces broken organisms whereas the freezing and drying technic yields homogeneous organisms.—*M. S. Marshall*.

DAVID, H. Über ein einfaches und sicheres Verfahren zur Geisseldarstellung. Zentbl. Bakt., I Abt. Orig., 132, 240-3. 1934.

This method, a combination of those of Gray and Zettnow, is carried out as follows: Inoculate 18-24-hr. agar slants and incubate for 6-8 days. Remove a loopful of bacteria from the upper part of the condensation water and place in a drop of tap water on a glass slide. After 1-2 min., remove a loopful of the slightly turbid drop and transfer to a second drop; 1 to 2 min. later spread a loopful of this drop, snake-fashion, over a flamed cover slip. Air dry and draw rapidly once thru the flame.

Place the cover slip in a fresh mordant soln. (mix 5 cc. 1:10 alum, 2 cc. 1:16 Hg Cl₂, and 2 cc. 20% tannin) for 10 min., wash under a light stream of water and dry by heating. Transfer to the following silver soln: Dissolve 5 g. AgNO₃ in 20 cc. dist. water, add to 6 g. NaSO₄ soln. in 10 cc. hot dist. water. Wash the ppt. quickly a few times with 20 cc. dist. water, suspend in 500 cc. water. Mix and store in a brown bottle unshaken. Heat to vapors and remove when the preparation browns or a white precipitate forms around the edge. Place under water and air dry, (not between filter papers). If necessary to bring flagella out more plainly, stain 2 min. with concd. carbol fuchsin. Mount in Canada balsam.—*J. A. de Tomasi.*

DE MEGNI, N. Ricerch sull' agglutinazione aspecifica con sostanze coloranti del gruppo dell' acridina. Boll. Ist. Sieroter. Milan., 13, 336-43. 1934.

Tripaflavin, chrysanilin, acridin orange and acridin red are used 1:500 in a 0.9% saline soln. at pH 7. In agglutination tubes 0.5 cc. of the culture are added to 0.5 cc. of the dye; the drop agglutination can also be used. Chrysanilin and acridin orange equal tripaflavin in its agglutination power, acridin red sometimes may lyse bacterial cells. "R" strains of the typhoid-coli-dysentery group agglutinate with any of these acridin dyes, "S" strains do not.—*J. A. de Tomasi.*

DI ATCHELBERG, U. L'agglutinazione aspecifica con la fucsina basica nella dissociazione batterica. Giorn. Batter., 14, 507-12. 1935.

Basic fuchsin in a 1:2000 diln. added to a 1:4000 suspension of bacteria proved to be an unusually sensitive reagent for the detection of bacterial morphological changes. The dye solution agglutinates promptly R type, but none of the S type organisms.—*J. A. de Tomasi.*

EVANS, F. L. Note on the fixation of smears for bacteriologic study. J. Lab. & Clin. Med., 20, 883. 1935.

Some bacterial material does not prove to stick to the glass after usual heat fixation. The author's modification is intended for difficult anaerobic spore-formers. The slide with the smear is held at 37° C. for 30-45 min., transferred for 10 min. to 170° C. in the hot air oven. If organisms are apt to multiply at 37° C., fix first at 55° C. for 15 min.—*J. A. de Tomasi.*

GEHM, H. W. and HEUKELEKIAN, H. Eosin methylene blue agar for rapid direct count of *E. coli*. Amer. J. Pub. Health, 25, 920-3. 1935.

This method has been found to be particularly adaptable to sewage plant control and sewage research. EMB agar is prepared according to the standard method, except that 2% instead of the usual 1.5% agar is used. Plates are poured to a depth of 3 mm. One cc. of inoculum is smeared over the surface and the plate placed in the 37° incubator to dry, with cover removed. The plate is dried for an hour, the inoculum being redistributed over the plate by tilting when about half dry. After the surface is thoroly dry the cover is replaced, the plate inverted, incubated at 37° for 24 hr. and characteristic colonies counted. Close agreement was obtained between this method and brilliant green bile broth tubes on various sewages. The method is cheaper, and quicker than other methods but comparable with them.—*M. W. Jennison.*

MEYER, K. F. and JOHNSTONE, H. G. Laboratory diagnosis of amebiasis. Amer. J. Pub. Health, 23, 405-14. 1935.

The method of choice in the laboratory diagnosis of amebiasis remains the preparation of wet-fixed, wet-treated, and hematoxylin stained permanent fecal smears. Method: 1. Smear is made with a paste brush on a clean flamed slide. 2. Without allowing slide to dry immerse immediately in the fixing fluid (Schaudinn's). 3. For fixation and staining the following methods should be used: Schaudinn's fluid heated at 60° or over night at room temp., 10 min.; 70% alc. tinged to a wine color with a satd. sol. of iodine in abs. alc. or with Lugol's soln., 10 min.; 70% alc., 5 min.; 50% alc., 5 min.; 35% alc., 5 min.; tap water (running), 2 min.; dist. water-rinse; 2% iron alum-aqueous soln. heated to 30°, 10 min.; tap water (running), 10 min.; dist. water-rinse; 0.5% hematoxylin-aqu. soln. heated to 30°, 10 min.; tap water (running), 5 min.; dist. water-rinse; differentiate in 2% iron alum heated to 30°; tap water (running) 20 min. 35% alc., 5 min.; 50% alc., 5 min.; 70% alc., 5 min.; 95% alc., 5 min.; 100% alc.,

5 min.; carbol xylol, 5 min.; xylol, 5 min. Mount with a thin layer of balsam thinned with xylol for temporary slides or with thicker balsam and cover slip for permanent slides.

Schaudinn's fluid. HgCl_2 , satd. aqu. soln. in normal salt, 2 parts; 95% alc., 1 part. This is the stock soln. and will keep indefinitely. On using add 4 cc. glacial acetic acid to 90 cc. of the stock soln. This soln. should be made up fresh at frequent intervals.

Hematoxylin stain. Stock soln.: hematoxylin, 10% in 100% alc. Cork, and ripen by standing in light for 3 months or more. Stain: hematoxylin, stock soln., 1 part; dist. water, 19 parts.

Iron-alum. Ferric ammonium sulfate crystals, 2% in dist. water. The crystals must be clear and purple or lavender, not yellow.

Carbol xylol may be used in place of abs. alc. if the absolute becomes contaminated with water; or in the handling of a large number of slides it may be used between abs. alc. and pure xylol. (Two washings of toluol may be substituted for the carbol-xylol and xylol.)

Carbol-xylol. Fill wide mouth bottle 1/3 full with clear white crystals of carboic acid, then fill rest of bottle with xylol. If crystals are yellow or brownish in color the preparation will be discolored.—*M. W. Jennison.*

MONNÉ, L. Permeability of the nuclear membrane to vital stains. *Proc. Soc. Exp. Biol. & Med.*, 32, 1197-9. 1935.

Vital staining of two *Amoeba* species was attempted, using some 40 dyes, and a technic of spraying the stain against the nucleus with a micro-pipette. Immersion of the *Amoeba* into dye solutions failed. In general, the more complicated dyes were more toxic. Vital staining of the nucleus was obtained with: 3 nitro dyes, 7 azo-dyes, 5 thiazins, 4 oxazins, 1 amido-azin, 1 safranin, 1 diamino-triphenylmethane, 4 triamino-triphenyl-methanes, 2 amino-hydroxyl-triphenyl-methanes, 1 pyronin, 1 rhodamin, 3 fluorane derivatives, 4 sulphonphthaleins, 1 acridin. Recovery occurred when the nucleus was partly stained with: water blue, azo blue, methyl blue, alizarin red, and ruthenium red. The following dyes always killed: sodium carminate, trypan red, nigrosin, vital red HR, rosindulin GG and congo red.—*M. S. Marshall.*

ONO, K. Studien über die Färbung der Rekurrenzspirochäten, insbesondere über den Zusammenhang der Färbung mit den Vorbehandlungen. *Acta dermatol. (Kyoto)*, 23, 73-95; 24, 1-42. 1934. (Abs. in *Zentbl. Bakt.*, I Ref., 119, 38-40. 1935.)

V. Einfluss der Vorbehandlung mit Kal. permanganat-Lösung auf die Farbbarkeit der Rekurrenzspirochäten:

Blood smears from rats infected with relapsing fever spirochetes of Manchurian stock are fixed in 1% KMnO_4 , thoroly washed, and stained 15 min. in 1% soln. of 282 dyes. Controls (as in all following papers) are fixed 15 min. in alcohol-ether before staining. After KMnO_4 , erythrocytes stain weaker; spirochetes and leucocytes stronger with basic dyes only; some of the acid dyes act better than others. Best results are obtained with 66 dyes, mostly of the triphenylmethane series, among which are named: ethyl violet, cerise, crystal violet, dahlia, extra violet, fuchsin, gentian violet, indol blue, indophene blue, magenta, marine blue, methyl violet, safranin, thionin, ultra blue and Victoria blue.

VI. Einfluss der Vorbehandlung mit Alaunlösung auf die Farbbarkeit der Rekurrenzspirochäten:

Fixation of spirochete blood for 15 min. in 5% alum is compared again with that in alcohol-ether. Erythrocytes stain weaker, spirochetes and leucocytes mostly stronger. Marine blue, methyl violet, new Victoria blue, and Victoria blue are named among the 29 best dyes.

VII. Einfluss der Vorbehandlung mit Karbollösung auf die Farbbarkeit der Rekurrenzspirochäten:

Fixation of spirochete blood smears with 5% phenol for 15 min. yields: stronger stain of erythrocytes and blood plasma, weaker stain of spirochetes and erythrocytes. Of the 282 dyes, 45 were satisfactory, e.g., ethyl violet, crystal violet, dahlia, gentian violet, and marine blue. Moreover, 24 dyes stained spirochetes and leucocytes after the phenol but not after alcohol-ether, e.g., acid magenta, benzo blue, benzo purpurin, diamine violet, eosin and patent blue.

VIII. Einfluss der Fixierung der Lösung des molybdansauren Ammoniums auf die Farbbarkeit der Rekurrensspirochäten:

After 15 min. fixation with 5% $(\text{NH}_4)_2\text{MoO}_4$, the erythrocytes take a weaker stain, the blood plasma remains colorless, the spirochetes and leucocytes stain stronger. Best suited are 47 dyes among which are named: ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, magenta, methyl violet, ultra blue, and Victoria blue.

IX. Einfluss der Fixierung mit Kal. bichromat-Lösung auf die Farbbarkeit der Rekurrensspirochäten:

After 15 min. fixation with 5% $\text{K}_2\text{Cr}_2\text{O}_7$, erythrocytes stain weaker, spirochetes and leucocytes much stronger. The blood plasma remains unstained. Among the 26 best suited dyes are: "baselin blau", crystal violet, gentian violet, sky blue, marine blue, methyl violet and Victoria blue.

X. Einfluss der Fixierung mit Mullerscher Flüssigkeit auf die Farbbarkeit der Rekurrensspirochäten:

After 15 min. fixation in Muller's liquid the erythrocytes stain weaker, spirochetes and leucocytes mostly stronger. The plasma does not take the stain. Among the 25 best dyes are: "baselin blau", dahlia, gentian violet, sky blue, marine blue, methyl violet, violet red and Victoria blue.

XI. Einfluss der Fixierung mit dem Orthschen Gemische auf die Farbbarkeit der Rekurrensspirochäten:

After 15 min. fixation in Orth's liquid, results are like those with Muller's fixative. Among the 39 best dyes are: ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, sky blue, magenta, marine blue, methyl violet, violet red and Victoria blue.

XII Die Farbemethoden der Rekurrensspirochäten:

Fixation is performed here for 5-15 min. with 10% formalin, or 3% H_2O_2 , or 1% KMnO_4 (also formalin followed by either of the other two). Staining time is reduced to 2-5 min. (instead of 15 min.) and the conc. to 0.2% (instead of 1%). All 3 fixatives give good results, altho it is hard to decide which to recommend owing to their respective distinct characteristics and advantages. Formalin-fixed smears stain well with ethyl violet, "baselin blau", crystal violet, dahlia, gentian violet, marine blue, methyl violet, and Victoria blue. Fixation in H_2O_2 proves even better, adding to the list several other dyes among which are sky blau and ultra blue. After fixation in KMnO_4 , staining succeeds with a great number of dyes in 2 min. and with 0.2% soln.—*J. A. de Tomasi.*

PETROFF, S. A. and GUMP, W. S. Bacteriostatic and bactericidal studies of various dyes and allied compounds. *J. Lab. & Clin. Med.*, 20, 689-98. 1935

A number of compounds with established bacteriostatic and bactericidal properties have completely failed to bring about desired results when used for therapeutic purposes. Since ordinary beef infusion media are unsuited for such a study, human serum agar was employed. The bacteriostatic property of some 130 dyes and allied compounds were studied on 10 Gram-positive and 7 Gram-negative organisms; the effective ones were selected for bactericidal tests. The list of organic dyes employed includes representatives of nitroso, nitro, azo, stilbene, triphenylmethane, xanthene, acridine, quinoline, azine, oxazine, thiazine and anthraquinone dyes. Plates were poured using pneumococcus agar of pH 7.6, cooled to 45° C. and added up to 10% with pleural fluid. Each Petri dish was divided in 2 halves by means of a strip of cardboard to be withdrawn after the control agar had solidified in one of the compartments. The second compartment received the serum agar plus the dye in dilutions ranging from 25,000 to 200,000 for Gram-positive organisms and from 5,000 to 100,000 for the Gram-negative. The dyes were first dissolved either in water or 95% alc. By means of rulings on the bottom, 6 or 7 streaks were possible across the 2 compartments of each plate, using 0.1 cc. of inoculum from an 18-hr. growth and a 3 mm. platinum loop. Incubation was set at 37.5° C. and results recorded after 48 hr.

Most of tested compounds proved to be of little or no value. The basic triphenylmethane dyes were most effective with the acridine dyes next in order.—*J. A. de Tomasi.*

SANDER, F. Bakterienfärbung durch Kaliumpermanganat; zugleich eine einfache Sporenfärbung. *Zts. Hyg. Infektionskr.*, 116, 335-6. 1935.

Clean the slide with ether and make a smear from a solid growth suspended in saline or dist. water. Air dry and fix in the flame. Flood for 2 min., preferably with a

cold satd. KMnO_4 soln. in 1% H_2SO_4 . Rinse, blot dry, and observe in oil. Owing to stronger oxidation of the membrane, bacteria appear stained yellow with a darker outer zone. In case of spores, follow same technic, but heat the oxidant for 2 min. on the slide. Vegetative cells stain yellow; spores, brown.—*J. A. de Tomasi*.

SANDER, F. 50 Jahre Gramsche Färbung und ihre Theorie. Zentbl. Bakt., I Abt Orig., 133, 385-400. 1935.

Following an ample review of the many diversified opinions as to the nature of the Gram stain, a broad analysis of conditions affecting the reaction was studied. Organisms of the coccus, bacterium, bacillus type, and yeasts were fixed on the slide by flaming and treated, previous to staining, with a number of reductants and oxidants. The action of hypertonic and hypotonic solutions of neutral salts as well as of non-electrolytes was also considered. Furthermore, suspensions of materials and the influence of the biological polar system of age and environment were similarly taken into consideration. The theory of condensation nuclei fo. the deposition of the stain was also tested.

Briefly, many reversible reactions were possible whereby Gram-positive organisms may be made Gram-negative and *vice versa*. The theoretical considerations under each of the chapters are developed at great length.—*J. A. de Tomasi*.

SYDENSTRICKER, V. P. and VRYONIS, G. P. Vital staining of malarial parasites. J. Lab. & Clin. Med., 20, 1094-6. 1935.

It is asserted that any technic that gives good reticulocyte staining will give satisfactory results with malarial parasites. Most satisfactory is the following: Place a small drop, approx. 2 mm., of sat. soln. of brilliant cresyl blue in sterile saline on a chemically clean slide; put a small drop of blood on a coverslip and drop directly on the stain. Ring with soft petrolatum. Staining is almost immediate, the parasites remaining alive for upwards of 2 hr. on the warm stage. Cytoplasm presents a coarsely stippled appearance, pale blue with slightly darker areas; chromatin is dark blue; pigment readily visible.—*J. A. de Tomasi*.

WILLIAMS, J. W. and GREEN, L. III. Effect of dyes on colonies of certain pathogenic fungi. Proc. Soc. Exp. Biol. & Med., 32, 625-8. 1935.

Microscopic and macroscopic observations were made with 27 fungi on a medium (4% peptone, 1% dextrose, 1.5% agar, pH 5.6) containing alcoholic nigrosin, litmus, eosin Y, and eosin B. To 5 batches of this basic medium were added, respectively: 2% fluorescein (Schultz No. 585, Lot No. 6054), 1% methyl blue (Lot. No. 3421) and 1% eosin Y (Schultz No. 587, Batch NE-7), 0.5% neutral red (Lot No. 7272), 0.5% Janus green (C. I. No. 133), 0.5% Wright's stain (NW-7). (These dyes were manufactured by the National Aniline and Chemical Company.) Growth was in diffused light at room temperature. Colonies showed characteristic colorations, and cellular elements were in some instances superior to specimens stained from growth on ordinary media.—*M. S. Marshall*.

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POLARIZED LIGHT TECHNIC

A COMPARISON WITH THE MARCHI METHOD

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ABSTRACT.—The authors compare polarized light technic for the study of degeneration of myelinated nerves with the Marchi method. While polarized light shows much the same thing, it has the advantages of being much more rapid, more sensitive, and constant. It does not depend on fixation and staining but on the chemical structure of the myelin substance. These changes in structure begin before the third hour after cutting the nerve of a rat. No further technic is involved beside the making of frozen sections. Photographs are presented to illustrate the method.

POLARIZED LIGHT TECHNIC

Most histological technics used with nervous tissues are slow, laborious, and often unreliable even in the hands of an experienced person. None of them at their best demonstrate the earliest and minute amounts of degenerative change in the nerve fibers. The method that has been most commonly used in the past has been that of Marchi. It has been assumed by most workers that this method gives fairly constant and reliable results and many studies of degeneration have been based on it. However, recent critical investigations of the method itself by Duncan (1931), Swank and Davenport (1935), and others, have shown that interpretations based on this technic are likely to lead to erroneous conclusions unless carefully controlled by other methods. Prickett (1934) has questioned its value in early or small amounts of degeneration and states that he found difficulty in distinguishing experimental from normal nerves in material where degeneration was supposed to be present.

Swank and Davenport (1935), state that four days after section is the earliest time that degeneration can be demonstrated by the Marchi method. Some of the methods used by Cajal and his students are said to show degeneration within 18 hours after cutting a

nerve. This means that the actual beginning of degeneration has not been demonstrated by any staining method at the present time. Thus it is evident that a more sensitive method is needed if we are to study the earliest stages of myelin degeneration as well as details of its progress. Preferably such a technic should not depend on the artefacts produced by fixation and staining but should make use of such chemical and structural changes as have occurred at the time of observation. This may be an impossible condition to expect to find. It is the purpose of this paper to present a method which approximates these conditions and which has already demonstrated degenerative changes within the first three hours after section of the nerve.

The chemical facts on which the Marchi method is based are well known. During degeneration the phospholipids of normal myelin are changed to triglyceride fats. In the latter state they are stained by osmic acid and are, therefore, identifiable as blackened particles in the sheath of the nerve fiber. But in addition to the triglycerides which accumulate during degeneration there appear to be somewhat similar substances produced by fixation, trauma, etherization, and time itself (Swank and Davenport, 1935). These substances stain with osmic acid and are extremely confusing in many cases and undoubtedly have led to erroneous conclusions in the past. These substances have been referred to as "dust" by some writers. Erlholz bodies are apparently another type of structure present in both normal and degenerating nerves. These also stain with osmic acid and still further obscure the actual changes in degeneration.

Polarized light offers a method of observation which we do not believe depends on artefacts and in which the usual artefacts are not visible if present. The pieces of nerves are placed for a minimum of 24 hours in 10% formalin for the purpose of hardening. We have not been able to see that fixing for this length of time in formalin changes the appearance of the fibers in the least. The nerves are then sectioned longitudinally on the freezing microtome at a thickness of 15 to 20 μ . These sections are floated from water onto slides and mounted in a drop of glycerin. Observations are made with a 4 mm. objective in polarized light and between crossed nicol prisms. Since normal myelin is birefringent, it will appear alternately light and dark four times in the rotation of the stage of the microscope. Any isotropic material, which includes degeneration products, is not birefringent and will appear dark at all points in the rotation. Thus any material within the limits of vision at this magnification can be identified as to the presence or absence of

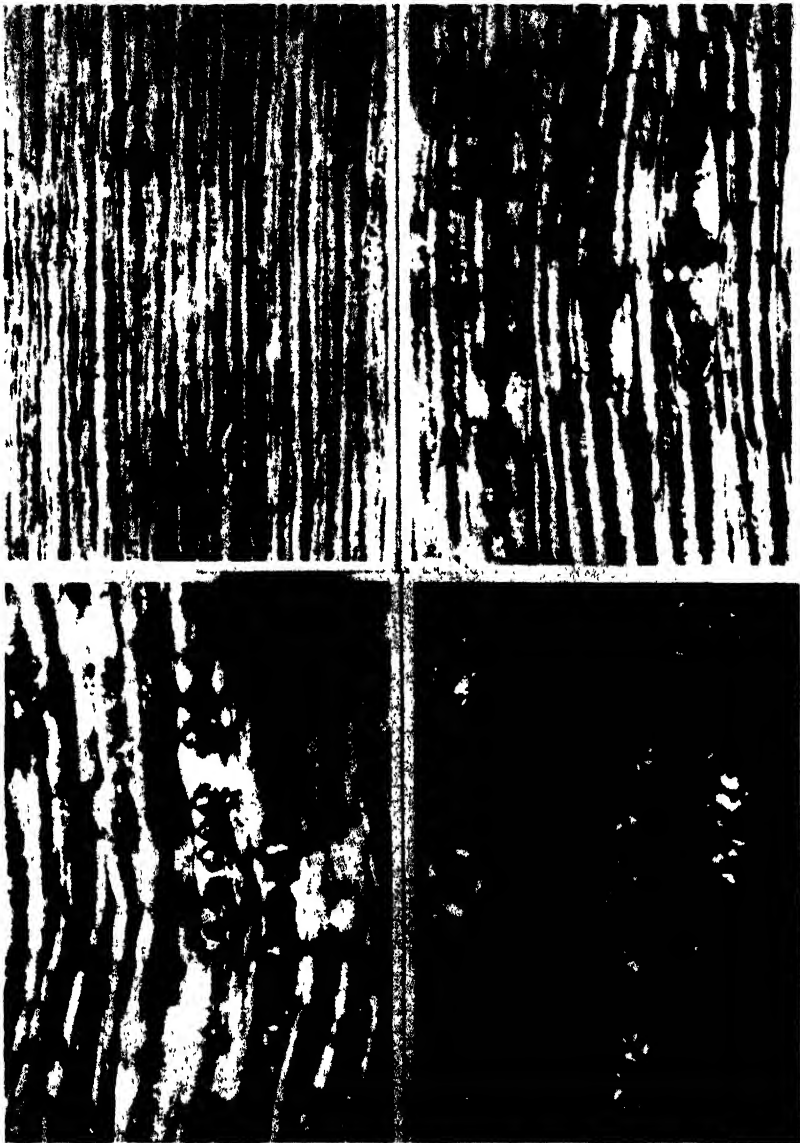


PLATE I

Explanation of Figures

All sections were photographed in polarized light and between crossed nicol prisms.

1. Section of a normal sciatic nerve (450x).
2. Section of a sciatic nerve three hours after cutting (450x).
3. Section of a sciatic nerve twelve hours after cutting (450x).
4. Section of a sciatic nerve twelve days after cutting (450x).

birefringence. Degeneration products begin to appear before the third hour after cutting the nerve as extremely fine granules, so small at first that the field appears cloudy instead of showing discrete particles. These rapidly become confluent during the first six hours so that definite droplets of isotropic fats can be seen in all the fibers. Even before loss of birefringence is apparent there is a very great swelling of the fibers which is plainly visible by this method, but is never seen in dehydrated material.

Degeneration products are not the only isotropic materials in a nerve. The sheath of Schwann, axis cylinder, the nuclei of Schwann cells, connective tissue cells, and certain cytological details of the structure of myelinated fibers are isotropic in normal as well as degenerating fibers.

Since these sections cannot be made permanent, photographs are the only permanent record possible. The photographs reproduced here are selected to show the early changes visible in three hours after sectioning the nerve, as well as more extensive ones occurring during several days. Details of the progress of degeneration in nerves of the rat have been described and published elsewhere (Setterfield and Sutton, 1935). Changes due to various pathological conditions, not degenerative in the usual sense, have been observed and will be published in the future. Also in another paper we will describe in some detail the problem of photography of nerve fibers in polarized light.

CONCLUSIONS

The method of polarized light is offered as a supplement to the Marchi and other methods for the study of myelin degeneration. It is simple, rapid, and accurate. It approximates the observation of unchanged nervous tissue and its sensitivity depends more on the skill and experience of the observer than on fixation and staining.

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A NEW METHYLENE BLUE TECHNIC FOR PERMANENT PREPARATIONS¹

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ABSTRACT.—1. Tissues stained *intra vitam* with methylene blue are fixed in a 10% ammonium molybdate solution in physiological saline (or sea water if the tissue is from a marine animal). Fixation time is kept to a minimum. Washing also is reduced to a minimum.

2. Excess fluids are removed from tissues by blotting with a paper or cloth towel before they are put into the succeeding solution. Tissues are taken from the wash water, blotted and placed in a mixture of equal parts of absolute ethyl alcohol and *n*-butyl alcohol for 30 minutes. They are then blotted and transferred to *n*-butyl alcohol for 30 minutes. After blotting they are placed in a mixture of one part methyl salicylate and four parts xylene until cleared. Tissues may be mounted whole or prepared for sectioning by embedding in paraffin in the usual way.

3. Tissues fixed, washed, dehydrated and cleared as described retain nearly all of the stain; the time required is greatly reduced; there is no need to chill the dehydrating solutions; cell distortion is much reduced.

The value of methylene blue as a vital stain has been considerably restricted by the difficulty of making satisfactory permanent preparations. A loss of stain during dehydration can be greatly reduced by the use of a modified ammonium molybdate solution recently reported (Cole, 1934) which fixes the dye more firmly in the tissues. On the other hand, the use of dehydrating solutions in which methylene blue is less soluble than in the usual ethyl alcohol series seems to be an alternate method. Zirkle (1930) found that *n*-butyl alcohol is of value in eliminating excessive hardening of plant tissues, and that it also facilitates dehydration and clearing of tissues (Zirkle, 1934). The following method, in which *n*-butyl alcohol is used, has given excellent results with vertebrate and invertebrate tissues stained *intra vitam* with methylene blue, when permanent preparations were required.

¹This paper is the second of a series covering work on methylene blue staining made possible by a grant from the Sigma Xi Society.

METHOD

Methylene blue² was dissolved in physiological saline solution in dilutions varying from 1:3,000 to 1:10,000, according to the tissue to be stained. Frog tissues, such as skin, skeletal muscle or enteric muscle tunic were treated either by injection or immersion. When the desired elements had been stained, the tissue was placed in an ammonium molybdate solution. This was freshly prepared, approximately 10% in concentration, and made up in physiological saline.³ The best fixation of methylene blue in the tissue was obtained with the minimum immersion in the ammonium molybdate solution, 30 minutes being adequate for small pieces or thin tissues.

The fixed tissue was then washed either in running water or in four changes of tap water for a total period of 30 minutes. Thruout the following steps it was important to remove excess fluid by blotting with a cloth or paper towel before the tissue was transferred to the next solution. After washing, the tissue was put into a mixture of equal parts of absolute ethyl alcohol and *n*-butyl alcohol for 30 minutes or more. It was found that ethyl alcohol alone removed appreciable amounts of the methylene blue, while *n*-butyl alcohol alone prolonged dehydration; the mixture, therefore, of equal parts of these two alcohols at room temperature was tried and proved to give the desired results. Following the bath in the alcohol mixture, the tissue was blotted and transferred to *n*-butyl alcohol alone for 30 minutes. With thick tissues it was usually more satisfactory to dehydrate further in a second bath of *n*-butyl alcohol, again for 30 minutes. It was found to be unnecessary to chill any of the alcohol solutions. After blotting the tissue was transferred directly to a mixture of one part of methyl salicylate and four parts of xylene. As soon as it was cleared, the tissue was mounted whole; or, if sections were desired, it was passed thru two baths of melted paraffin, one hour in each, then embedded in the usual way. Sections were cut from 20 to 40 μ in thickness; since the tissue was nearly transparent and the nerve fibers could be studied to advantage in thick sections. A droplet of celloidin-clove oil fixative (equal parts of thick celloidin and of clove oil) was applied to a slide and rubbed to a thin film. Sections were laid lightly on a slide thus prepared, and

²Two separate lots of methylene blue were used, both National Aniline products and both certified by the Commission on Standardization of Biological Stains.

³The need for careful weighing in the preparation of each fresh solution can be eliminated by the use of "beetleware" measuring spoons procurable at any ten-cent store, and by the computation of the amount of fluid required for approximately a 10% solution.

oriented. They were covered with non-porous paper and gently patted into contact with the fixative. This method permits immediate immersion in xylene. Following removal of the paraffin from the sections, dammar or balsam was added and a coverglass applied.

Study of these preparations reveals that the stain is retained, distortion of cells is slight, and tissues are not excessively hardened. The method is equally satisfactory for frog skin or enteric muscle tunic and for more delicate tissues such as the skin of the squid.⁴

SUMMARY

1. Tissue stained *intra vitam* with methylene blue retains practically all of the dye when *n*-butyl alcohol is used in the dehydrating solutions in accordance with the method described.
2. The time required for dehydration is greatly reduced by the use of equal parts of ethyl and *n*-butyl alcohol.
3. Chilling the dehydrating solutions is unnecessary; these solutions at room temperature extract but little stain.
4. Distortion and rupture of cells is greatly reduced by shortening the time of both fixing and washing, and the tissue is not hardened excessively.

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⁴In staining and fixing marine animals, sea water or a saline solution isotonic with it was used.

AN IMPROVEMENT IN STAINING TECHNIC FOR PROTOZOA¹

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ABSTRACT.—A modification of Donaldson's iodine-eosin stain for staining intestinal protozoa is presented. This modification consists of using high dilutions of colloidal iodine (Chandler)² instead of Lugol's solution as well as high dilutions of eosin. A better resolution of the external and internal structures is brought about by the new method.

The procedure is as follows: A portion of the fecal material to be examined is suspended in a 0.6% salt solution; the suspension should be of a consistency so that one drop will make a satisfactory microscope mount under a cover glass. To ten parts of this suspension, in a test tube, is added one part of the stain which is prepared as follows:—

10 parts of distilled water

6 parts of a suspension of colloidal iodine (Chandler) containing
4% iodine—20% iodine suspensoid, Merck

1 part of a 10% water solution of anilin red, Merck (eosin yellowish)

Technicians will find, because iodine in the form of colloidal iodine is readily released to the organisms, that the use of this material is far superior to Lugol's solution in carrying out the technic for staining intestinal protozoa in the study of fresh mount preparations. Not only are organisms more deeply stained with iodine but by eosin as well, even when employed in high dilutions.

Donaldson's iodine eosin stain³ (one part Lugol's solution to one part of a saturated water solution of eosin) has been used for a number of years as a stain for intestinal protozoa in fresh mount preparations. Apparently the function of the Lugol solution is that of killing the organism, thus permitting penetration of the eosin. This, however, gives a uniform red stain with no appreciable degree of

¹Approved for publication as Journal Article No. 242 (n.s.) from Mich. Agric. Exper. Station.

²Chandler, W. L. Iodine as a disinfectant against nematode eggs and larvae. Proc. U. S. Live Stock Sanitary Assoc., December, 1925.

Chandler, W. L. and Miller, E. J. Colloidal iodine. J. Phys. Chem., 31, 1091-6. 1927.

See also Merck's Index, 1930 edition.

³Donaldson, R. An easy and rapid method of detecting protozoal cysts by means of wet-stained preparations. Lancet, 192, 571. 1917.

differentiation. The endosome and flagellae are seen vaguely, if at all. Apparently the iodine in Lugol's solution is not liberated in sufficient amounts to stain internal structures with iodine, the potassium iodide in the solution being a stronger solvent of the iodine than the organism.

Protozoa are readily killed and stained with suspensions of colloidal iodine (Chandler) and staining of internal structures and flagellae are especially marked, the degree of staining depending upon the strength of the suspension and the length of application. In colloidal iodine suspensions, water is the only solvent present; hence, the iodine in solution readily passes into the organism which has a greater affinity for iodine. Suspended particles of iodine present in excess of that required to produce a saturated water solution quickly go into solution, replacing that which was taken up by the organism.

The transfer of iodine from water solutions to the organism and replacement by dissolution of suspended particles continues until the iodine-fixing power of the organism is satisfied, or until all of the iodine is used up; or, until the organisms are removed from the suspension. Colloidal iodine alone, however, does not bring about a sufficient degree of internal differentiation to permit of satisfactory diagnostic studies in all cases.

If equal parts are used of a saturated water solution of eosin and colloidal iodine, containing an amount of iodine corresponding to that in Lugol's solution (thus simulating the Donaldson stain technic) flocculation of the material in the fresh microscopic mount occurs and prevents accurate observations of the specimen.

If, however, as was recently observed by the writer, higher dilutions of mixtures of both anilin red (eosin yellowish) and colloidal iodine are used, no flocculation occurs and a very marked degree of differentiation is obtained thruout all the structures of the protozoan. The endosome within the nuclei of amoebae, the nuclear membrane with its thickened and thinned portions, as well as the circular and radiating fibrils within the nuclei of certain species of flagellates, are clearly defined.

It seems highly proper that this little discovery be passed on to those who are interested. It is not necessary, however, that a detailed account be given here of the experimentation done with this stain in working out the most effective combinations of stains and dilutions. Suffice it to say that as a result of the above observations, researches were conducted in the Laboratory of Parasitology at Michigan State College and satisfactory combinations and dilutions were worked out.

The writer prepared a series of combinations of the stains which, when applied to the genus *Chilomastix*, revealed a remarkable degree of staining of both internal and external structures. The stains used consisted of a combination of eosin (various forms) and colloidal iodine in high dilutions. The percentage of eosin was unknown, as old materials were used.

Efforts were made to discover the exact combinations of these two stains necessary to bring about the most satisfactory results. A fresh sample of "eosin yellowish" (water soluble) was obtained and a large number of mixtures of various percentages of each component were prepared and applied to both fresh and preserved specimens of several genera of parasitic protozoa, including amoebae, flagellates, and coccidia. Thru careful observations and a process of elimination, correct percentages of each stain were discovered and a stain developed which gave results even more effective than the original combination.

After the formula was determined, it became the purpose of the writer to resolve the facts into simple but accurate directions for the use of this stain which can be readily followed in any biological or hospital laboratory. The first step is to collect and preserve the fecal sample. The technic as developed by Dr. W. L. Chandler is as follows:

The patient should be instructed, or the physician advised to instruct the patient, to drink a one-half pint bottle of citrate of magnesia just before retiring. The next morning about eight o'clock or so, regardless of the number of evacuations during the night, the patient should be instructed to collect a stool sample directly into a pint or one-half pint fruit jar and without urine contamination. This sample should reach the laboratory within one-half hour after being voided.

The laboratory technician should heat to body temperature about 50 cc. of a 0.6% salt solution in a small Erlenmeyer flask, and a sufficient amount of the stool sample should be immediately thoroly fragmented in this warm salt solution, using a sufficient amount so that a drop will make a microscope mount that readily can be studied.

If the stool sample is solid or semi-solid, the transfer to the salt solution can be made by means of a mushroomed glass rod, making as many transfers as is necessary to bring about the desired suspension. If the stool sample is liquid, it can be transferred to the salt solution by means of a medicine-dropper, or pipet provided with a medicine-dropper bulb.

The salt solution suspension of the fecal material should then be iodized by introducing as rapidly as possible, drop by drop, colloidal iodine containing 4% iodine, using enough so that a faint iodine color is still present at the end of two minutes. The saline suspension should then be filtered thru cheesecloth. This can best be accom-

plished by using a small beaker into which to filter the material. The cheesecloth should be wet with water in order to facilitate filtration. To this filtered material should be added an amount of formalin sufficient to make a 5 to 10% formalin solution. The material can then be transferred to a bottle for shipping or stored for further observation. Materials collected and preserved in this manner have remained in good condition at this laboratory over a period of years.

The object of the warm saline solution is to encourage motility of the organisms, then if the organism is immediately killed, which can be done by the use of colloidal iodine, the motile characters are preserved. As an example, if motile *Chilomastix* species are killed slowly they have a tendency to round off and the principal characteristics are lost; also if amoebae with extended pseudopodia are killed slowly they retract these pseudopodia. If the iodization is properly done the organisms are killed almost instantly and the typical characters of motile forms may be observed in the preserved material.⁴

To ten parts of such a preparation add one part of the following stain mixture:

- 10 parts of distilled water
- 6 parts of a suspension of colloidal iodine (Chandler) containing 4% iodine.
- 1 part of 10% water solution of eosin yellowish⁵

The ten parts (drops or cc.) of water should be placed in a glass stoppered bottle, the colloidal iodine added and the mixture agitated; and then the eosin Y solution added and the mixture again agitated.

This stain combination apparently does not deteriorate with age, but should be thoroly shaken before use. It can be used for immediate study of fresh preparations or stained preserved material can be kept indefinitely. The degree of differentiation of the organism's structure is increased for a period of ten days after adding the stain and then remains in a satisfactory condition permanently.

⁴Laboratory instruction sheets, Bacteriology 307, Michigan State College. 1935.

⁵Merck's eosin yellowish employed by writer.

THE PHYSICAL CHEMISTRY OF SILVER STAINING

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ABSTRACT.—It has been shown that silver deposition plays a part in the silver staining process. From this it has been concluded that the rate of reduction of silver within and on histological structures is an important factor.

Some factors controlling the rate of reduction, such as the adsorption of silver hydroxide and ammonia, the affinity of silver for proteins, and the protective power of the gel structures have been pointed out.

Some simple applications of the ideas to silver staining have been given and two technics described, one making use of piperidine instead of ammonia, the other carrying out the reduction in the presence of the silver solution to facilitate deposition.

HISTORICAL

Cajal (1903) believed that tissues contained albumens which united with silver and that the resulting silver compounds could be easily reduced, thus making structures visible. In 1920 he made the suggestion that in silver ammino-carbonate solutions an "ammoniacal oxide" of silver is produced which is taken up by the histological elements in a selective manner. He gave no data, however, on this point.

Bolsi (1927) presented a view that was essentially an elaboration of Cajal's idea. He believed that the silver salts are selectively adsorbed by tissue elements.

Liesegang (1911) suggested that Cajal's stain is analogous to the photographic process. He drew the analogy in the following manner: While the tissue is in the silver nitrate solution, by virtue of some reducing action of the histological elements, some silver is reduced, and this forms the nuclei as in the exposed photographic plate; upon subsequent treatment with hydroquinone these nuclei have silver deposited upon them and outline histological elements.

Cajal (1921) discussing Liesegang's theory, accepted the principle of the "deposition" character of the stain but offered two objections to the nucleus idea. The first was that treatment of tissues with oxidizing agents did not change the staining; the second, that the theory did not explain what he called the "transference of nucleation". Thus, as he pointed out, when tissues were treated with various reagents, different structures were stained.

Kubie (1929) suggested that when tissues are placed in the reducing bath following immersion in silver ammino solutions, silver salt tends to diffuse out and, depending upon the speed of reduction, silver will be deposited either in the tissue or upon its surface.

Visintini (1931) thought that the reduction reaction forms a silver sol and that this sol is then specifically flocculated upon histological elements.

THE DEPOSITION NATURE OF THE STAINING PROCESS

It is the aim of this paper, first, to show that an essential feature of the staining process is the deposition of silver upon the stained elements and, second, to discuss some of the physical and chemical factors influencing this deposition. Before proceeding with this, it is necessary to discuss briefly the chemical compounds used.

The Chemistry of the Silver Ammino Compounds. Reychler (1895) showed that when ammonium hydroxide is added to silver nitrate, first silver hydroxide is precipitated and then, as more base is added, the silver hydroxide redissolves to form a compound $\text{Ag}(\text{NH}_3)_2\text{NO}_3$. The exact amount of ammonia required to make a clear solution is given by various authors: Herz (1910) gives 2.12 moles of ammonia for each one of silver; Kubie and Davidson (1928), 2.06 at 25°; Euler (1903), 2.08.

The state of equilibrium in a solution of silver diammino-nitrate is given by Kohlschutter and Fischman (1912) as follows: The salt ionizes completely into a complex cation $\text{Ag}(\text{NH}_3)_2$ and the nitrate anion; the complex cation dissociates slightly giving silver ions and by hydrolysis ammonium and hydroxyl ions; the excess of ammonium hydroxide above 2 moles for each one of silver is necessary to depress the number of silver ions so that silver hydroxide will not be precipitated. A complete diagram of this equilibrium is given by Kubie and Davidson (1928).

Euler has pointed out that the compounds formed by silver nitrate with methylamine and ethylamine are analogous in every way with that formed with ammonium hydroxide. It is assumed that piperidine acts in the same way as these amines. These amines are stronger bases than ammonia, and the equilibrium conditions of their complex silver salts are different from that of silver diammino-nitrate. Table 1 gives the dissociation constants of the complex cations, calculated from the pH, as done by Euler.

These figures indicate that the compounds formed with ethylamine

TABLE 1. DISSOCIATION CONSTANTS

Base used to form amino-compound with AgNO_3	Molarity of silver solution	Ratio of base to silver in moles	pH measured with indicators	K calc. from pH	K calc. from silver electrode
Ammonia	.05	2.09	9.9	1.3×10^{-8}	1.5×10^{-8} (Euler)
Ethylamine	.05	2.20	10.9	1.0×10^{-7}	2.5×10^{-7} (Euler)
Piperidine	.01	3.60	11.8	5.4×10^{-6}	No data

and piperidine will behave differently from the ammonia compound in regard to their reduction, adsorption, diffusion, and possibly, therefore, also in their staining properties. Other amines which form complex silver compounds such as diethylamine, *n*-butylamine, ethylenediamine were tried in stains with less success. The long chain amines separate into an oily phase and cannot be used.

Experimental Proof of Deposition. If it can be definitely shown that a large fraction of the metallic silver present on or in a stained element is deposited during the reduction, then the simple view advocated by Cajal and Bolsi must be altered.

To each solution in Table 2 was added 0.5 g. of raw Japan silk fibers. After 2 hours aliquot samples of the silver solutions were withdrawn and titrated in nitric acid with ammonium thiocyanate, using ferric ammonium sulfate as an indicator. From this titration the amount of silver taken up by the fibers was calculated. To each flask 0.5 cc. of 37% formaldehyde solution was added. The time for reduction to start, as indicated by the presence of reduced silver, was noted. After two hours the fibers were removed and washed with large quantities of distilled water until all the coarse particles of silver mechanically held in the meshes of the fibers were removed. The fibers were then washed in sodium thiosulfate to remove any unreduced silver salts and again in distilled water. The metallic silver staining the fibers was dissolved in concentrated nitric acid and titrated. Table 2 shows that in all but two cases the amount of silver finally present was greater than that held adsorbed before the reduction. We must conclude then that during the reduction process metallic silver is deposited upon and in the protein fibers.

If the fibers are removed from the silver solution and added to a formalin solution, the results are different. The staining becomes a race between the process of reduction and the process of diffusion. Using 10% formalin it was noticed that the fibers from all the solutions stained well, except the fibers in solution No. 4. Here, as can

be seen from Table 2, the reduction was slow so that silver diffused away from the fibers before reduction could occur. Similar experiments, using frozen sections of brain tissue in place of fibers, showed that 1.3 to 2 times the adsorbed silver was finally present in the tissue. Here the frozen sections were removed from the silver bath and reduced one by one in 5% formalin.

A simple histological observation upholds the deposition idea. Clear areas about deeply stained nuclei as in Fig. 3 (Plate I) are often seen. It may be noted that axis cylinders which come close to these nuclei are unstained, indicating mobilization of silver.

TABLE 2. DATA INDICATING DEPOSITION OF SILVER ON FIBERS

Content of solution 50 cc. volume	Ad- sorbed silver (grams)	Silver found on fibers	Time for reduction to begin	Character of solutions after re- duction	Color of fibers after re- duction
1) 4.9 cc. .1N AgNO ₃ 4.9 cc. 2.1N NH ₄ OH Contains .052 g. Ag.	.010	.028	10 min.	Clear with brown tint	Black
2) 4.9 cc. .1N AgNO ₃ 4.9 cc. 2.3N ethylamine Contains .052 g. Ag.	.015	.033	5 min.	Slightly brown, no precipitate	Black
3) 4.9 cc. .1N AgNO ₃ 4.9 cc. 3.6N piperidine Contains .052 g. Ag.	.022	.022	Immediate	Black with precipitate	Brown
4) 4.9 cc. .1N AgNO ₃ 9.8 cc. 2.1N NH ₄ OH Contains .052 g. Ag.	.010	.032	30 min.	Clear	Black
5) 4.9 cc. .1N AgNO ₃ 4.9 cc. .05N Na ₂ CO ₃ 4.9 cc. 2.1N NH ₄ OH Contains .052 g. Ag.	.011	.030	3 min.	Brown	Gray
6) 4.9 cc. .1N AgNO ₃ 4.9 cc. .1 N NaOH 9.8 cc. 2.1N NH ₄ OH	.021	.017	Immediate	Black with precipitate	Brown- yellow

The mechanism of deposition in the formation of mirrors has been studied by Kohlschutter and Fischman. They showed that reduction of silver goes on rapidly at surfaces because of the strong adsorption of both formaldehyde and silver hydroxide. As silver is deposited more silver hydroxide is adsorbed and reduced. We see, therefore, that the faster the reduction goes on at the surface in relation to the reduction in the body of the solution, and the more time given for diffusion to take place to the surface, the greater the deposition will be. The results shown in Table 2 agree with

this conception. In the case of the fibers, the slower the reduction the more deposition. In solutions 3 and 6, Table 2, the solutions surrounding the fibers were so rapidly reduced due to the high pH that no time was allowed for diffusion to the fibers. In tissue staining, as in the Bielschowsky technic, there will be a race between reduction and diffusion, so that if the reduction is too slow, no staining takes place.

Summing up, we can say that deposition must play a part in staining and that the deposition depends upon the rate at which the reduction reaction occurs on and within histological structures.

FACTORS WHICH INFLUENCE THE RATE OF REDUCTION

Since the deposition depends upon the rate of reduction at the surfaces of the histological elements, we are interested chiefly in those factors which are operative at these surfaces rather than in general factors, such as temperature. A brief glance at the general nature of the reduction reaction is first necessary.

When formaldehyde is added to silver diammino solutions we should expect a quantitative formation of hexamethylenamine, since free ammonia is present. This should lead to the formation of silver hydroxide which could react with formaldehyde still present. At the same time formic acid is produced. The conditions are so complex that we cannot in any way predict which of the above reactions will proceed more rapidly. In general, there is an excess of formaldehyde present in silver staining so that the removal of formaldehyde from a solution in the form of hexamethylenamine need not worry us.

Measurements by the writer indicate that the time course of the above reaction is given by a sigmoid curve, indicating a catalysis. Some experiments by Kohlschutter and Fischman indicate that the metallic silver formed may be the catalyst.

The presence of reducing substances in tissues which might form catalytic "nuclei" of silver has been demonstrated by Loew and Bokorny (1881) Masson (1928), Kon (1933), and others. It might be thought that it would be easy to manipulate these nuclei by means of the well-known procedures of photography and thus determine their influence. As has been pointed out by Cajal, the results of such a manipulation are inconclusive. We can only add that if such nuclei are formed in tissues, they must be highly protected by the gel structures and are, therefore, of secondary importance. The autocatalytic nature of the reduction will tend, however, to accentuate any differences in the initial rates of reduction.

The adsorption of silver and formaldehyde. From Table 2 it may be noted that silver is removed from solutions by protein fibers. It is important to know in just what form silver is removed.

Silver ammino solutions were allowed to stand two hours with samples of wool yarn. The silver adsorbed was determined by titration of an aliquot sample. The carbonate was measured in the Van Slyke apparatus. The total base adsorbed was determined by titrating an aliquot sample with nitric acid, using methyl orange as an indicator. The ammonia was determined by adding strong sodium hydroxide to the aliquot sample and distilling it into a measured amount of standardized acid.

Experiment I			Temp. 24°C.
Solution: Silver diammino-carbonate		Ag. conc. molarity	CO ₂ conc. molarity
Before adsorption	No. 1	.095 M	.05 M
	No. 2	.090 M	.044 M
After adsorption	No. 1	.026 M	.049 M
	No. 2	.050 M	.042 M

Similar results were obtained when silver ethylamine carbonate was used.

Experiment II				Temp. 23°C.
Solution: Silver diammino-nitrate				
Adsorbent	Initial conc. Ag. sols.	Millimols Ag. adsorbed	Millimols NH ₄ adsorbed	Millimols Base adsorbed
0.5 g. wool	.02 M	.51	.18	.71
0.5 g. wool	.02 M	.51	.175	.70
0.5 g. dry brain tissue	.02 M	.24	.16	.37

Experiment III				Temp. 23° C.
Adsorbent	Amine used to make complex with silver nitrate	Millimols Ag. adsorbed	Millimols base adsorbed	pH
0.5 g. wool	Ethylamine	0.58	1.48	10.8
0.5 g. wool	Piperidine	0.74	1.68	11.7

From these figures one may conclude: (1) that the anion is not taken up (Exp. I), (2) that ammonium hydroxide and silver hydroxide are adsorbed since the base equals the silver plus the ammonia (Exp. II), (3) that the ratio of silver to base varies as different compounds are used (Exp. III). Since base may buffer the formic acid produced by oxidation of formaldehyde, and since ammonia may react with adsorbed formaldehyde to form hexamethylenamine, the

ratio of base, amine, and silver must play an important role in controlling the rate of reduction of silver. The conditions are so complex that it is impossible except by experiment to tell what the optimum ratios for deposition are.

The writer has studied the adsorption of formaldehyde upon silk and wool fibers. As no definite equilibrium is reached in several days, no exact figures can be given. The adsorption is great, however, for in low concentrations large fractions of the total formaldehyde disappear from the solution. This is consistent with the finding that surface active agents, such as ethyl alcohol, saponin, and butyl alcohol, do not greatly inhibit the deposition of silver in such experiments as were presented in Table 2. These agents are thus unable to displace formaldehyde from the protein fibers.

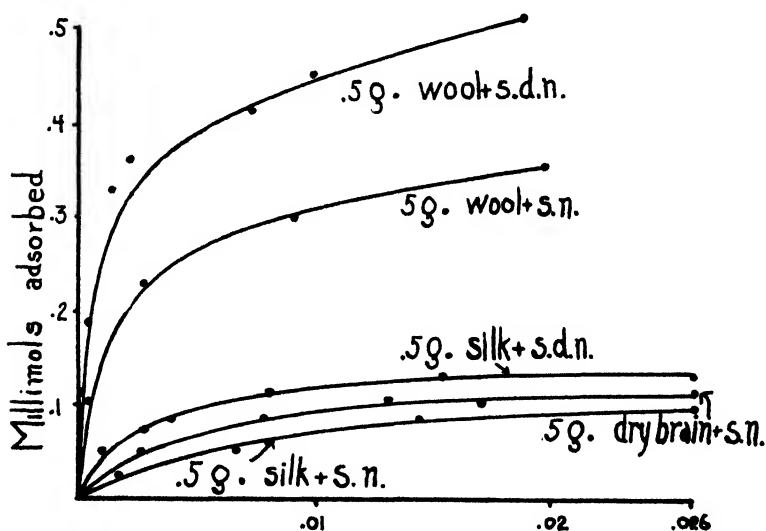


Fig. 1. Equilibrium molarity s.n.=silver nitrate
s.d.n.=silver diamminonitrate

Since the majority of commercial samples of formaldehyde contain large percentages of methyl alcohol and also some formic acid, fresh solutions of formaldehyde were made by dry distillation of paraformaldehyde. These solutions were standardized according to the method of Romijn (1897). In the staining of tissue only these solutions were used.

The influence of concentration and the affinity of silver compounds for fibers. It might be at once expected that the amount of silver compound taken up by the structures would influence the rate of reduction. The amount taken up depends upon the concentration

in the surrounding solution, as may be seen from Fig. 1. The results, when plotted upon chained coördinates, gave straight lines. This indicates that the union follows the Freundlich adsorption isotherm.

We may reason as follows: Adsorption of silver occurs only because a decrease in free energy takes place. As the concentration increases, the adsorbent becomes saturated and successive increments of adsorbate cause less free energy change and are held less avidly. The rate of reduction, therefore, of adsorbed silver compounds should increase rapidly as the concentration of the silver solution increases.

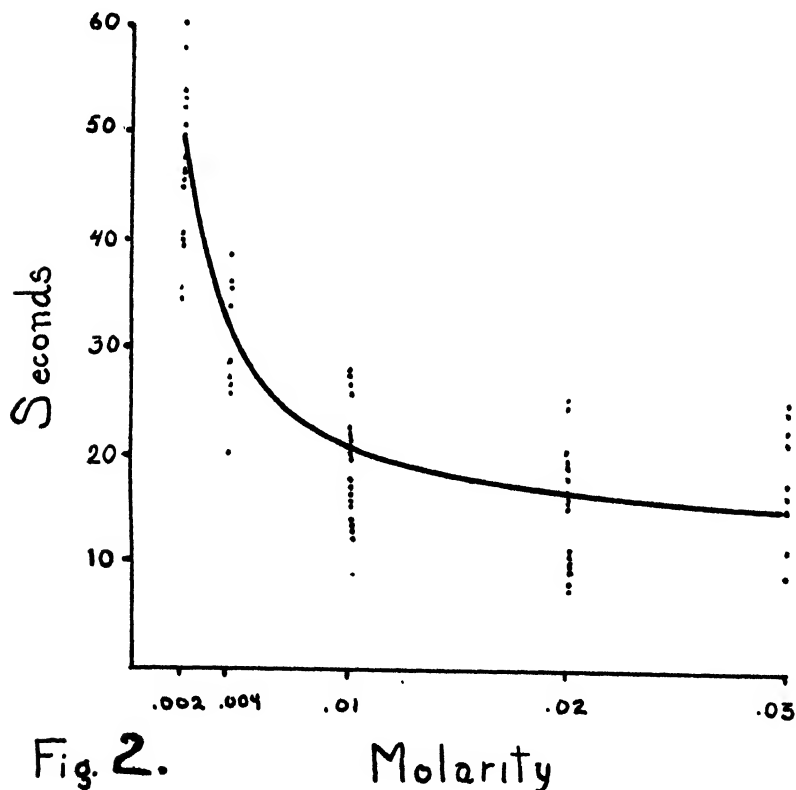


Fig. 2.

Molarity

This idea was tested in the following manner: Silk fibers were immersed in varying concentrations of silver diammino-nitrate. The fibers were removed from the silver bath and placed upon a microscope slide, the excess solution being removed with a piece of filter paper. A full strength solution of formalin (37%) was then dropped upon the fibers. The fibers were watched with the low power of the microscope and the time for the occurrence of the first definite brown

color was measured with the stop watch. The results of such an experiment are given in Fig. 2. The curve plotted by inspection shows the trend. It is in agreement with the above idea.

Control experiments show that between molarity of 0.002 to 0.05, reduction occurs, i.e., a brown tint appears in the solutions in from 25 to 35 seconds and is independent of dilution. This is what might be expected when the reacting agent in these ammoniacal solutions is silver hydroxide. When dilution occurs the amino-compound undergoes greater hydrolysis, and the amount of silver hydroxide remains almost the same.

From Fig. 1 it may be seen that at lower concentrations wool fibers are relatively less saturated than silk fibers. We expect then that at these concentrations the staining of wool would be more difficult than that of silk. At saturation concentrations we should expect that the staining would become about the same for the two types of fibers, for the affinity of the last increments of silver would approach zero in both cases. Experiment shows, however, that in concentrations of 0.03 M, i.e., close to saturation (Fig. 1), silk fibers are stained while wool fibers remain entirely white, altho they contain about four times the silver of the silk fibers. The staining was done by transferring the fibers from the silver solutions without washing into 1% formalin. Upon this basis we may conclude that the difference in staining properties between silk and wool depends upon some factor other than their affinities for silver compounds.

The role of protective action. Reinders and Van Nieuwendurg (1912) showed that protective colloids inhibit the reduction of silver nitrate by ferrous citrate. Williams and MacKenzie (1920) found that gelatin retards the precipitation of silver dichromate for many hours. In both cases the retardation is ascribed to the slowing of the rate of crystallization. Because of this influence the precipitation of an insoluble substance occurs more rapidly in one gel than in another.

If we add to 5 cc. of a 0.5% solution of iso-electric gelatin, 0.5 cc. of 0.05 normal silver nitrate and then add 0.3 cc. of 0.05 molar potassium bichromate, silver dichromate will be precipitated in several hours. If silk and wool fibers are added to this solution, some fibers take on a distinctly red color long before the surrounding solution becomes tinted red by silver dichromate. Microscopic examination shows that the silk fibers are stained deep red by a very fine and even deposit of silver dichromate. The wool fibers are colored a light yellow and have apparently taken up potassium bichromate. The interpretation is as follows: The gelatin cannot diffuse into the fibers. The salts can do so easily. The protective power of silk fibers

being less than that of the surrounding gelatin solution, the precipitate starts within these fibers sooner than in the surrounding solution. This precipitate once formed grows by diffusion. The protective power of the wool fibers being greater than that of the surrounding solution, no precipitate is formed. Differences in density do not explain the difference in staining, for in this case there should at least be a precipitate upon the surfaces of the wool fibers. In carrying out these experiments with other samples of gelatin it may be necessary to vary the concentrations given. The important point is that the precipitate should form slowly over a period of hours.

A frozen section of formalin fixed brain tissue was placed in a 2% silver nitrate solution for 24 hours. It was then washed with distilled water and placed in a test tube containing 3 cc. of 1% gelatin solution. To this solution was added 0.04 cc. of 0.1 molar silver nitrate and 0.2 cc. of 0.1 molar potassium dichromate. The test tube was left over night in an incubator at 37°C. Fig. 4, Plate I, shows a section stained by this method. Nerve fibers are clearly and sharply shown, altho they are not completely stained. There is no diffuse coloring of the tissue.

Upon the above basis and from the results of the silver staining experiments, we can assume that nerve fibers in company with silk fibers stain easily with silver because their gel structure allows precipitation of metallic silver to occur readily. A simple observation supports this view. It can be noted in silver stained sections that the colors assumed by tissue elements are exactly those that may be seen in silver sols of different particle size. Nageotti and Gyon (1930) have shown by observation with the cardioid condenser that silver stained structures contain colloidal particles of silver. Von Weimarn (See Gortner, 1929, p. 14.) has shown that the degree of dispersion of a sol is dependent upon the ratio of the rate of nuclear formation to the rate of crystal growth. Since the gel structure can affect the rate of crystal growth the above color variations are exactly what should be expected, if each structure has a different protective power.

The conception of the Golgi stain based upon the above considerations is as follows: The brain substances form a non-homogeneous gel. When the tissue is immersed in potassium dichromate it takes up a certain amount of the chromium salt. The silver nitrate diffusing in produces a supersaturated solution of silver dichromate. Precipitation begins in the regions where protective power is least. More silver dichromate diffuses to those regions and is added to the precipitate already present.

Many facts uphold this theory. When liver is stained by the Golgi method, the bile canaliculi often stand out sharply. This is exactly what we would expect since the canaliculi presumably being spaces, would fill up with precipitated silver dichromate, while the cells being protective gels would have only a finely dispersed precipitate. Frequently the capillaries of the brain are shown beautifully outlined. Careful examination shows that the structures seen are solid rods of silver dichromate precipitate. The lumen of the capillaries is filled up in the same manner as the bile capillaries.

PRACTICAL APPLICATIONS

If it is true that silver staining is at least in part a process of deposition, then the most favorable conditions for staining should occur where no diffusion away from the tissue is possible and where diffusion to the tissue is possible. This condition occurs if we carry out the reduction process in the presence of the silver compound.

The section shown in Fig. 5 (Plate I) was stained by the following procedure: The frozen section of formalin fixed tissue was placed in a test tube containing 1 cc. of 0.05 molar sodium carbonate, 1 cc. of 0.1 molar silver nitrate, and 1 cc. of 0.209 molar ammonium hydroxide. To this was added 0.05 cc. of 0.08 molar formaldehyde solution freshly made from paraformaldehyde. The tissue gradually became dark while the surrounding solution remained clear.

In the procedure just described staining occurs long before any colloidal silver is produced in the solution surrounding the frozen section. The silver sol must, therefore, be produced in such intimate relation to the tissue that no silver particles diffuse away. The staining cannot then be analogous to immersing tissue in sols, and the particle size does not determine what structures are stained, as held by Visintini (1931) but, on the contrary, the structures determine by their properties the size of the colloidal particle produced within them.

The above process is so simple that if standard solutions are made up we can repeat the staining in identical fashion time after time. When frozen sections from a large series of brains, all supposedly fixed in an identical manner, are stained, wide variations are seen. Fig. 6 is an example of a result often obtained. The nuclei stand out black upon a bright yellow background showing no detail. This section was stained together with one from the material shown in Fig. 5 as a control. Fig. 7 shows a section taken from the same block as the section in Fig. 6, stained after 4 days' additional fixation in fresh 2% formaldehyde. Fixation in 10% silver nitrate for several days often causes the same change. Fixatives not only

PLATE I

Fig. 3 is a frozen section of formalin fixed white matter from the cerebrum. The section was stained by the Bielschowsky technic, using silver ammino-nitrate and sodium hydroxide with a 5% formalin solution as the reducing agent. The clear areas about the nuclei are thought to be due to a mobilization of silver from the surrounding areas. $\times 200$.

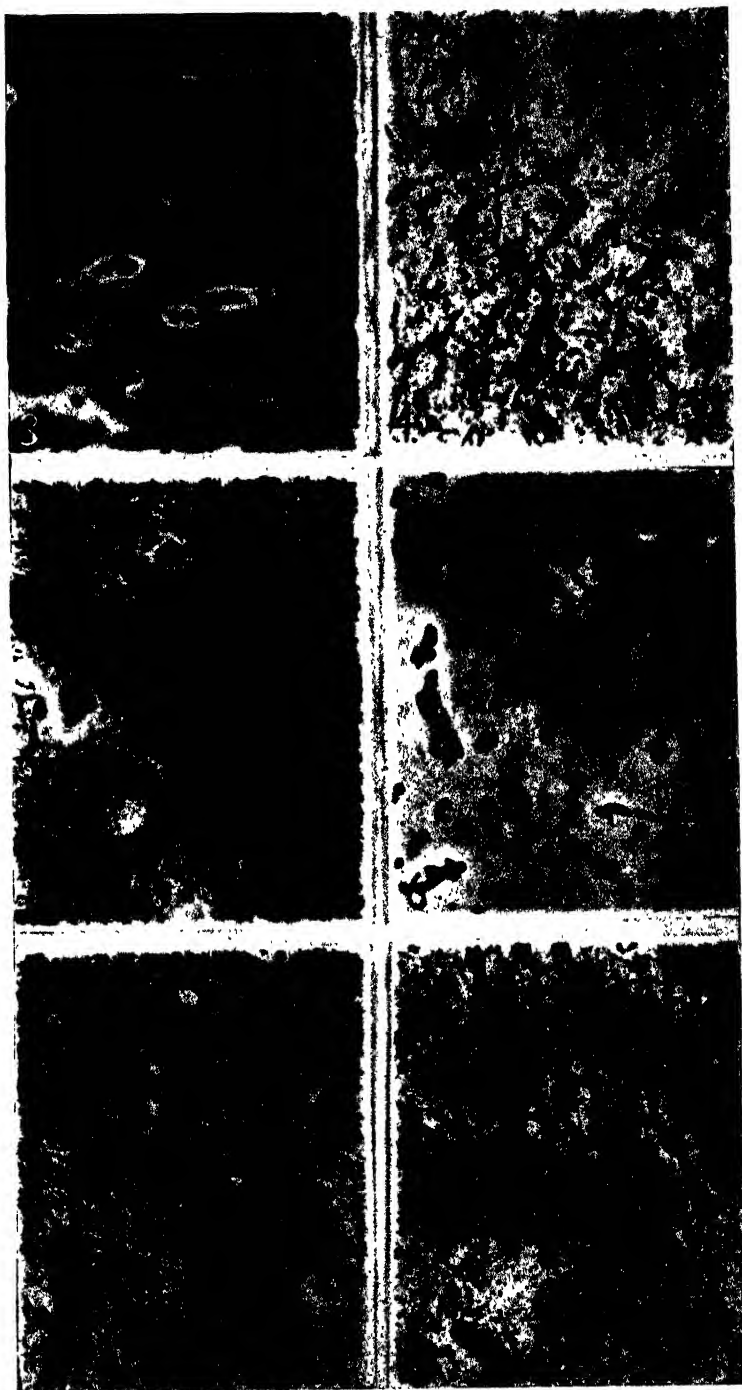
Fig. 4 is a frozen section of formalin fixed white matter stained with silver dichromate as described. This section shows that the silver dichromate is deposited upon the nerve fibers but nowhere else. $\times 200$.

Fig. 5 is a frozen section of formalin fixed white matter from the cerebrum. The section shows the sharp staining of fibers obtained by the modified procedure of allowing the reduction to take place in the silver solution. $\times 200$.

Fig. 6 is a frozen section of formalin fixed white matter from the cerebrum. The same staining procedure as in Fig. 5 was used. The nuclei stain deeply while the nerve fibers are light yellow with no detail. The yellow areas appear as white in the photograph. This section represents a not uncommon result obtained with silver stains. $\times 200$.

Fig. 7 is a frozen section from the same material as shown in Fig. 6. The frozen sections were allowed to remain in a 2% formalin solution for four days before staining. The staining procedure was the same as in Fig. 5. The section shows a marked contrast to Fig. 6 in that the nerve fibers are sharply and accurately stained. $\times 200$.

Fig. 8 is a frozen section of gray matter from the same material as Fig. 7, stained with piperidine silver carbonate as described. This is a fairly typical result obtained with this stain and shows the peripheral glia and several microglial cells as well as the pyramidal cells of the cortex. $\times 300$.



change the protective power of gels but also their ability to adsorb silver. It can only be indicated here that the fixation and condition of the tissue is probably the most important variable in silver staining.

It was indicated before that when the silver solutions are made up with ethylamine and piperidine, the ratio of silver and base adsorbed is different from that when ammonium hydroxide is the base. We must assume that different histological structures are affected differently by this change in adsorption so that there may be a differential effect upon staining as well as a general one. Fig. 10 is a frozen section stained by the following procedure: To 1 cc. of N/10 silver nitrate was added 1 cc. of 0.05 molar sodium carbonate and the precipitate was dissolved by the addition of 0.2 cc. of piperidine. The frozen sections were washed first in distilled water, allowed to remain in the silver solution from 1 to 3 minutes, and transferred without washing to 50 cc. of 0.05 molar formaldehyde solution.

Upon the basis of all the foregoing considerations we can offer the following conception of the Bielschowsky type of stain: The tissues take up ammonium hydroxide and silver hydroxide in various ratios. Each histological structure has a different affinity for the silver and also a different protective power. In the reducing solution adsorption of formaldehyde takes place and, depending upon the above factors, reduces silver hydroxide either slowly or rapidly. Those structures in which reduction of silver hydroxide proceeds fastest will acquire a deposit of silver from the solution carried over mechanically from the silver bath. This mechanically held silver will tend to diffuse away and if the reduction is too slow, will be unavailable for deposition.

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NOTES ON TECHNIC

STAINING WITH SAFRANIN AND FAST GREEN FCF.—Until within the last few years, the visual advantages of a red-green differential stain to bring out vascular tissue in contrast to non-vascular tissue have been offset by the fact that no permanent green has been available. The introduction of fast green FCF, which is an apparently permanent stain,¹ has made possible staining schedules giving red for xylem, and green differentiation of the other tissues, with a minimum of difficulty and a maximum of differentiation.

The following schedule is recommended by the writer for general use. It produces excellent slides, which have good visual contrast as well as excellent differentiation. We have used it to stain series of sections for floral anatomy, miscellaneous stems, leaves, and roots, which had been preserved in a variety of fluids. Some of the graduate students have tried it on celloidin material, and on wood sections, and have secured good results.

The following stock solutions are made up beforehand:²

- A. Safranin, 1% in 50% alcohol. (This is used diluted, 20 drops per Coplin jar, or about 2 cc. stock to 40 cc. tap water. Use once.)
- B. Fast green FCF, 0.5% in absolute alcohol.

STAINING SCHEDULE

1. Remove paraffin and hydrate to 70% alcohol. Xylol, 5 minutes; rinse in equal parts xylol and absolute alcohol; absolute alcohol, 5 minutes; 95% alcohol, 5 minutes; 70% alcohol, 5 minutes.
2. Put 20 drops of stock safranin into each Coplin jar. Add sufficient tap water so that the slides will be covered. Discard this solution after it has been used.
3. Place slides in safranin and stain. The time may be varied and naturally will vary with the material. Thirty minutes should be considered a minimum; 1 or 2 hours is enough in many cases; while if it is more convenient the slides may remain in the stain during the day or overnight without overstaining.
4. From this point on handle the slides 1 or 2 at a time. Rinse in 70% alcohol; rinse in 95% alcohol; rinse in absolute alcohol 5 to 15 seconds.
5. Dip the slide into a staining jar containing 0.5% fast green in absolute alcohol. (The fast green may be used in 95% alcohol

¹Haynes, Rachel. Fast green, a substitute for light green SF yellowish. *Stain Techn.*, 3, 40. 1928.

²A National Aniline safranin (Cert. No. NS-7) and a Coleman and Bell fast green FCF (uncertified) were used by the writer.

and the schedule readjusted.) Move the slide gently to and fro 6 to 10 times, or until it is properly differentiated.

6. Rinse in absolute alcohol.
7. Dip slide in clove oil, and move it to and fro 6 to 10 times in order to get rid of the alcohol.
8. Rinse in xylol to remove the clove oil. Check slide under microscope to be sure the stain is right.
9. Clear in xylol.
10. Put on cover glass.

—JOHN ADAM MOORE, Henry Shaw School of Botany, Washington University, St. Louis, Mo.

A NEW PROCEDURE FOR THE FEULGEN REACTION; A PRELIMINARY NOTE.—The Feulgen stain (see Feulgen and Rossenbeck, *Zts. Physiol. Chem.*, **135**, 203–48, 1924) has an established value in cytology, but is not in common use, partly because much of the basic fuchsin on the market is unsatisfactory, and partly because of difficulties in the technic. Attention is here called to certain changes in the technic which will be found of assistance.

- 1) Use of $K_2S_2O_5$ in dye solution and in acid rinse solution.
- 2) Use of distilled water thruout.
- 3) Increase in timing of steps to following:
 - 4–5 min. hydrolyzation
 - 2 hr. staining for animal tissue; 3–5 hr. for plant tissue.
 - 10 min. each in three successive baths of acid potassium bisulfite.
- 4) Use of fast green FCF or orange G instead of light green as counterstain.

By paying attention to these points and by taking care to use a batch of basic fuchsin showing decolorization in the Feulgen formula to a light straw color or faint pink, one can avoid many of the pitfalls of the technic. A later paper will discuss the procedure in more detail and give definite instructions as to how to obtain a suitable basic fuchsin.—J. A. DE TOMASI, Geneva, N. Y.

PRESENT MEMBERS OF THE COMMISSION ON STANDARDIZATION OF BIOLOGICAL STAINS

Altho it is only two years since the list of members of the Stain Commission was last published (April, 1934), there have been so many recent changes in personnel that publication of an up-to-date list seems desirable again. The names below constitute the membership of the Commission, as of March 1, 1936.

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LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

BOOK REVIEWS

PICKWORTH, F. A. **Chronic Nasal Sinusitis and Its Relation to Mental Disorder.** 7 x 10 in., 136 pp. Cloth. 83 illustrations. Index. Bibliographies. H. K. Lewis & Co., London. 1935.

This book is a comprehensive study of the subject under consideration. The text is supplied with a rich selection of photomicrographs and colored plates. Of special interest here is the "Appendix" because of some of the histological methods included, to wit:

Gram-positive organisms in tissues: Cut 10 μ paraffin sections (or 15 μ trichloroacetic decalcified bone). Run down to water. Stain 5-15 min. in 1% aq. eosin. Wash 5 min. in water. Rinse in alcohol. Stain 15-30 min. in freshly made and filtered anilin methyl violet: 2% aq. methyl violet, 9 cc. and 20% anilin in abs. alcohol, 1 cc. Wash with Gram's iodine and hold therein 2-5 min. Blot and clear in several changes of anilin-xylene. Wash in xylene and mount in neutral Canada balsam or resin.

Gram-negative organisms in tissues: Treat sections overnight in 10% H_2SO_4 . Wash 30 min. in running water. Stain 10-15 min. in Leishman's solution diluted 1:3 with dist. water. Wash in dist. water. Differentiate until pink in 1:1,500 acetic acid. Wash 3 min. in running dist. water. Stain 30-60 min. with Giemsa diluted 1:15 with 1% aq. pyridine. Wash in dist. water. Differentiate carefully in 1:3,000 acetic acid. Transfer to a mixture of equal parts of 1:200 tannic acid and 1:1,500 acetic acid. Wash in water. Dehydrate in acetone, clear and mount. Do not confuse organisms with occasional pigment granules of nerve cells or particles of corpuscular stroma of blood-clot.

Decalcification of sections containing bone: Cut thin (5 mm.) and treat (5 days to several weeks) with 5% trichloroacetic acid in saline. Formalin fixation does not interfere. Abs. alcohol may hasten the process.

Stain for vessels in thick sections: Cut fresh brain fixed within 6 hr. after death into slices 1 cm. thick. Fix 2 days in formol saline: NaCl, 20 g.; 40% formaldehyde, 100 cc; water, 1,000 cc. Wash 24 hr. in water. Soak in gum phenol: gum arabic, 500 g.; phenol crystals, 20 g.; water 2,000 cc. (Warm to dissolve, filter thru wool and Seitz filter.) Cut 250 μ frozen sections. Wash 2 hr. in running water. Transfer for 1 hr. at 37° C. to sodium nitroprusside benzidine: sodium nitroprusside (nitroferrocyanide) 0.1 g.; 0.5% benzidine in 2% acetic acid, 25 cc.; dist. water, 75 cc. Agitate the sections continuously in a shaking machine. Filter and use immediately. Rinse in water. Shake 1 hr. (37° C.) in dil. H_2O_2 : 20 vol. H_2O_2 in 400 cc. water. Wash in water, dehydrate, clear and mount. An additional cellular stain such as dilute Giemsa may be introduced before clearing.—*J. A. de Tomasi.*

THE MICROSCOPE AND OTHER APPARATUS

ANGERER, K. and HEYER, E. **Ein Lichtfilter zum leichteren Auffinden der nach Ziehl-Neelsen gefärbten Tuberkelbazillen.** *Munch. med. Woch.*, 82, 348-9. 1935.

A light filter is now available, made by Reichert (Vienna) upon the authors' suggestions, which helps to detect acid-fast bacilli. It is a combination of a yellow and violet filter which can be inserted beneath the Abbe condenser like any usual

blue glass filter. It will not work with daylight illumination. Ziehl-Neelsen stained acid-fast organisms stand out in ruby-red against a beige colored background. Other objects are bright green. (*Abstr. in Zentbl. Bakt., II Abt., 92, 506. 1935.*)—*J. A. de Tomasi.*

DE ZEEUW, J. and KUENEN, D. J. Note on a micro-colorimeter and its possible application. *Protoplasma*, 23, 626-9. 1935.

A simple device is prepared by mounting a semi-circular silvered cover slip at 45° in place of the fixed diaphragm in a microscope ocular. A 1 cm. hole is bored thru the side of the eyepiece opposite the mirror. In this way a horizontal light beam coming thru colored filters can be seen simultaneously with the object on the microscope stage.—*R. Chambers.*

RAMSTHALER, P. Ueber ein Mikroskopzusatzsystem mit Aperturblende. *Zts. Wiss. Mikr., 51, 184-7. 1934.*

All systems of side illumination as well as dark field condensers are at a disadvantage when compared with schemes of direct lighting and opaque illuminators. It is not possible to modify the aperture of the objective by means of a variable diaphragm. The author has developed an optical addition which allows projection at a suitable point within the microscope of both the virtual image of the object and the image of the frontal lens diaphragm. Here an iris diaphragm is built in, acting as an aperture diaphragm, which must be centered in each microscope to the image of the front lens diaphragm of the objective. The system is on the market as "Schrägblickaufsatz mit Irisblende" by Reichert, Vienna.—*J. A. de Tomasi.*

SCHARRER, B. Ueber die Feststellung von Dickenunterschieden an lebenden mikroskopischen Objecten, dargelegt am Beispiel der Unterscheidung von Hühner- und Rekurrensspirochäten. *Arch. Protistenkunde, 85, 87. 1935.*

Dark-field microscopy affords a highly sensitive means for distinguishing organisms that differ very slightly in thickness. With immersion condensers of high N. A., objects of the same thickness as *Spirochaeta recurrentis* appear double-lined. With condensers of lower N.A., i.e., dry condensers, the objects appear single-lined. Thicker objects appear double-lined even with dry condensers; thinner objects appear single-lined even with condensers and objectives of the highest N.A. Of a large number of spirochaetes examined none had the dark-field optical properties of *S. recurrentis*, as most of them were thinner.—*S. H. Hutner.*

UBER, F. M. Illuminator for critical microscopy utilizing automobile head-light lamps. *Science, 82, 624-5. 1935.*

Critical illumination for microscopy with high power objectives requires a small light source of uniform and high intensity. This can be obtained by focusing the light from a 6-8 v. headlight lamp on a finely ground glass used as a secondary source. In cases where the beam is insufficient for low-power field illumination, a supplementary lens may be inserted below the substage condenser. The intensity and size of the light beam can be easily varied by lowering a glass screen operated synchronously with the diaphragm.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

IVES, R. L. A simple method of determining areas in microphotographs. *Science, 82, 445-6. 1935*

Mechanical integration methods for determining areas in photomicrographs are slow and laborious. This method is based upon the principle that the weight of the photographic paper carrying the image of an object varies directly with the area of the image. Proceed as follows: Cut out of the print the image of the entire field (F) and weigh it. From the image of the entire field cut out the image (S) whose area is desired and weigh it. The desired area AS is given

by the equation $AS = \frac{WSAF}{WF}$ where WS and WF are the two weights determined

and AF is a numerical value obtained by integration. If the cutting is done carefully with a sharp blade, errors can be kept within 1%.—*J. A. de Tomasi.*

ROBINSON, W. L. A simple photomicrographic camera. *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, 13, 67-70. 1934.

This camera was designed to meet the needs for a recording apparatus in a routine laboratory. It is small, compact and as inexpensive as compatible with good performance. It is intended to be fixed permanently to the worktable along with the microscope and light. It consists of a box of seasoned white basswood, rectangular at its base, to accommodate 4" x 5" plate holders. Toward the top the box expands posteriorly to allow for a viewing aperture thru which focusing is done. There is also installed a right angle prism and a mirror set at 45° to deflect the image onto the base board at the bottom of the camera box. Focusing is similar to that of a Graflex camera. The main limitation is due to the fixed length of the bellows extension—a feature introduced for the sake of rigidity and simplicity in design.—*J. A. de Tomasi.*

DYES AND MICROTECHNIC IN GENERAL

GAILLARD, P. J. and HECHT, E. Ein Beitrag zur Vereinfachung gewebezüchterischer Kulturmedien. *Protoplasma*, 23, 1-13. 1934.

A dried petrol ether (ligroin) extract of pig's brain, after thoro extraction with acetone, furnishes a powder which, in very low concentrations, takes the place of embryonic juice for coagulating the plasma in tissue cultures. The authors maintain that this method is as good as the usual one.—*R. Chambers.*

LISON, L. Sur la détermination du pH intracellulaire par les colorants vitaux indicateurs. "L'erreur métachromatique." *Protoplasma*, 24, 453-65. 1935-36.

The author lists a series of metachromatic dyes from which he selected neutral red, brilliant cresyl blue, cresyl violet and Nile blue as examples of basic dyes which are used frequently as pH indicators (ionic virage) and which also shift color in the presence of chromotropic substances (metachromatic virage). These dyes were prepared in a concentration of M/10,000 in Sørensen's buffer solutions diluted to 1 part in 5 parts water. The pH color reactions of brilliant cresyl blue, cresyl violet and Nile blue agree with those stated by Fauré-Fremiet but not with those by Spek. The author also prepared similar solutions of the dyes in McIlvaine's buffer solutions from pH 2.2 to 8.0 (disodium phosphate, citric acid) diluted 1:6, to which was added a given amount of calcium chondroitin sulfate (prepared according to the procedure of Sawjalow). Other chromotropic substances were also used, e. g., agar-agar, the jellied extract of *Chondrus crispus*, cellulose, glycogen, gum arabic and chitin. It is significant that all these substances had no effect on the pH colors of Clark and Lub's indicators, or the universal indicator, B.D.H. For brilliant cresyl blue, with calcium chondroitin sulfate in concentration of 1/20,000, the metachromatic color, in solutions of pH 2.0 and 3.0, correspond to the color of pure buffer solutions at pH 10.4 to 11.4. Cresyl violet and Nile blue give similar metachromatic errors. Neutral red in the presence of 1/20,000 calcium chondroitin sulfate gives, at pH 2.2 to 6.5, the colors characteristic for pH 7.0 to 7.4.—*Author's Abstract.*

MAYERSON, H. S. Standardization of photochemical methods for the measurement of solar ultraviolet radiation. *Amer. J. Hyg.*, 22, 106-36. 1935.

The bleaching of acetone-methylene-blue solutions is not an ideal method for measuring solar radiation, but it is the simplest. The reaction depends on the decomposition of an aq. solution of acetone by ultraviolet radiation. It is partly an oxidative process and is accompanied at the same time by reduction processes which are measured by the addition to the solution of methylene blue which acts as a hydrogen receptor. The bleaching of the latter is proportionate to the time of exposure. The English standards as proposed by Webster, Hill and Eidinow (*Lancet* 206, 745-7, 1924) were duplicated as follows: Standard solution No. 10 (matching the standard acetone-methylene-blue solution) was made by adding 1 cc. of dist. water to each 2.4 g. of C. P. copper nitrate, previously dried in an electric oven for 24 hr. at 60° C., and filtering. Standard solutions No. 9 to No. 5 were made by diluting solution No. 10 with 0.05, 0.15, 0.30, 0.65 and 1 cc. of dist. water, respectively, for each cc. of solution. For standard solutions No. 4 and No. 3, solution No. 10 was diluted per cc. with 2 and 5 cc., respectively, of a 0.01% solution of K₂CrO₄. Duplicate sets of standards were prepared and

sealed in glass tubes of 3 mm. internal bore, and when not in use kept in the dark. Standard methylene blue solutions (No. 10) have also been prepared using 9 cc. of a 0.1% solution of medicinal methylene blue. (Schultz No. 659, National Aniline and Chemical Co.), or 15 cc. of a 0.1% solution of De Haen methylene blue 2B, diluted with 30 cc. of Merck's C. P. acetone and made up to 100 cc. with dist. water. The solutions completely absorb all radiations shorter than 305 $m\mu$ and absorb about 90% of the radiation shorter than 320 $m\mu$.

For the test, standard solution No. 10 is exposed vertically to the sun in a standard quartz tube of 55 mm. length and 3 mm. internal diameter, and the amount of fading measured by comparison with a set of standard tubes made up as previously described.

The method yields only very approximate values even under the most carefully controlled conditions. A glass control tube should always be exposed simultaneously with the quartz tube to measure the amount of bleaching in the visible spectrum. This amount should be deducted from the total bleaching under quartz. If the bleaching is too rapid, the tubes should be changed every 4 hr. Temperature corrections should be applied.—*G. H. Chapman.*

POPE, M. N. A bath for orienting objects in paraffin. *Science*, **82**, 356. 1935.

An embedding bath is described which will keep the paraffin melted until orientation is complete and will allow it to harden rapidly without change of position. It consists essentially of an open copper box with inlet and outlet pipes at opposite corners controlled by suitable stopcocks. The box is mounted horizontally on a tripod stand, and its inlet connects with a funnel held vertical above it. A copper sheet bent to reach the bottom of the box makes a platform on which paper trays with melted paraffin and objects can be kept warm. About 250 cc. of hot water in the copper box can be easily replaced with as much ice water, and hardening will occur in about 1 min.—*J. A. de Tomasi.*

RICHARDSON, G. D. An improved rapid celloidin method of embedding. *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, **13**, 81. 1934.

This method is intended to eliminate shrinkage of the sections. Cut tissue no more than 2 mm. thick. Carry out all the following steps in a water bath at 55° C. Fix 2 hr. in 10% formalin. Dehydrate 2 hr. in acetone. Clear $\frac{1}{2}$ to 2 hr. in clove oil. Infiltrate 3 hr. in celloidin. Block and harden in chloroform $\frac{1}{2}$ to 2 hr. The celloidin is prepared as follows: add 1100 cc. abs. alcohol to 8 oz. air-dried celloidin and allow to stand over night. Add 1100 cc. ether and hold until dissolved.—*J. A. de Tomasi.*

SCANLAN, J. T. The magenta series. I. The preparation and spectrophotometric study of the lower basic members. *J. Amer. Chem. Soc.*, **57**, No. 5, 887. 1935.

An attempt to correct some of the variations of basic magenta (fuchsin) was made. It was found that this stain contains different compounds such as intermediates that are undesirable. An improved method of preparation for two of the lower homologs was devised. Spectrophotometric methods were used to identify and evaluate the commercial samples studied.—*Roy L. Mobley.*

STEWART, M. J. A concentration method for the demonstration of asbestos bodies in the sputum. *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, **13**, 70-1. 1934.

About 15 g. of sputum are dissolved in an equal amount of antiformin by holding at 37° C. and shaking. From 60-90 cc. of water are added and the mixture allowed to stand a few hours. All but 12-15 cc. of the supernatant liquid is then decanted and the residue is moderately centrifuged for 10 min. After decanting and draining, the deposit is mixed thoroly but gently with a fine pipet and transferred to albuminized slides. Usually the whole sediment from a 15 g. sample may be spread over an area of 1 sq. inch. Dry on hot plate, fix over a flame, wash gently in water. Dry and mount in Canada balsam. Before mounting, the film may be treated with $K_2Fe(CN)_6$ and HCl to demonstrate the Prussian blue reaction given by the asbestos bodies; or, when black, they may be bleached with 5% $(NH_4)_2S$.—*J. A. de Tomasi.*

SYMEONIDIS, A. New uses of the cold-knife frozen-section method for fixed tissue. *Zentbl. allg. Path.*, 63, 245-6. 1935.

With the knife cooled below freezing, better frozen sections can be obtained from fixed as well as unfixed tissue. Fixed tissue sections tend to come off the slide, hence, should be applied to an albuminized slide, smoothed out, and blotted carefully with several thicknesses of dry filter paper or filter paper moistened with 50% alcohol. Transferring the preparation to abs. alcohol for 30-60 sec. coagulates the albumin and tends to prevent separation of the section. Subsequent processing can be the same as for paraffin sections. Special stains (e. g., fat) or the oxidase reaction can be applied after fixing sections for 1 min. in 40% formalin.—*H. A. Davenport.*

THORNTON, H. R. and SANDIN, R. B. Standardization of the methylene blue reduction test by the use of methylene blue thiocyanate. *Amer. J. Pub. Health*, 25, 1114-7. 1935.

Following the Standard Methods of Milk Analysis, 3 samples of methylene blue were tested in milk: standard American reductase test tablets of methylene blue (chloride), Danish tablets (also the chloride), and methylene blue thiocyanate prepared by the authors. Considerable variation in color depth were found even between different tablets in the same lot. Methylene blue thiocyanate was much more constant. The inadequacy of present empirical dye standards is due largely to the difficulty of purification and determination of purity of methylene blue. The following recommendations are made: (1) That methylene blue thiocyanate be substituted for methylene blue chloride in the standard tablets used in the methylene blue reduction test because of the reproducibility of the former dye; (2) That one part of dye to 300,000 parts of milk be adopted as the standard concentration in this test.—*M. W. Jennison.*

ANIMAL MICROTECHNIC

BERBLINGER, W. and BURGDORF. Neue Färbemethode zur Darstellung der Gewebbestandteile der Hypophyse des Menschen. *Endokrinologie*, 15, 381 8. 1935.

The new technic described for the demonstration of the various components of the hypophysis consists of the following steps: Fixation in 4-10% aq. or alc. formalin; hardening and dehydrating in graded alcohols; chloroform; paraffin. The sections, after removal of paraffin, are stained for 2-24 hr. in an alc. solution of cresofuchsin (0.5-1%), depending upon the strength of the stain, washed briefly in dist. water; then in alum-carmin for 3 hr.; rinse in dist. water, differentiate and stain the acidophil cells for 5 min. in the following: 100 ml. of 1% phosphomolybdic acid solution containing 2 g. orange G.; rinse in dist. water; place in 5% phosphomolybdic acid for 2 min.; dry with blotting paper; stain for 10-20 min. in (dist. water 100 ml., 0.5 g. water soluble anilin blue, bring to boil, filter cold and dilute filtrate with 2 parts dist. water); rinse in dist. water; differentiate in 75% alcohol until stain no longer issues from section; dehydrate; xylol, balsam. Chief cells, blue to gray; pregnancy cells, blue with small vivid yellow granules; basophiles with coarse reddish-blue granules; epithelium of the pars intermedia and pars tuberalis variable; collagen fiber, intensive blue, glia fibers, blue-gray; axons occasionally nearly black.—*J. M. Thuringer.*

COVELL, W. P. and O'LEARY, J. L. Vital staining methods applied to nerve degeneration. *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, 13, 92-3. 1934.

Neutral red was found to be specific for altered myelin. It can be used in various ways: intravenously, subcutaneously, by perfusion, and immersion. The intensity of the reaction depends upon the amount and concentration of the dye. A teased piece of a peripheral stump in 12 min. gives optimum staining in a 1:10,000 dilution of the dye. The extent and degree of degeneration can thus be quickly ascertained.—*J. A. de Tomasi.*

DUPRÈ, G. C. Un nuovo metodo di colorazione tipo Mallory. *Monit. Zool. Ital.*, 46, 77-83. 1935.

Fix in Zenker's with formalin or acetic, or preferably in Ruffini III (Muller's fluid, 50 cc.; 1% chromic acid, 50 cc.; glacial acetic acid, 2 cc; corrosive sub-

limate, 1 g.). After Ruffini III remove bichromate with Kaiserling II, adding several drops of 25% KI. Embed in paraffin or celloidin-paraffin. Cut sections 5 to 8 μ thick. Stain with Ziehl's basic fuchsin prepared according to Gallego, or when the fixing fluid contains acetic acid, use the following formula: Basic fuchsin, 1.5 g.; water, 160 cc.; 95% alcohol, 40 cc.; allow to stand 48 hr. at 37° C. before using. To 15 cc. of anilin water add 10 drops of the dye and 5-7 drops of acetic acid. Stain from 1-10 min. and pass into the following solution: water, 50 cc.; formalin, 20 cc.; glacial acetic acid, 25 cc. for 5-10 min. Wash briefly in water and pass into 1% phosphomolybdic acid for 10 min. Wash thoroly in water for about 2 min. Counterstain with any one of the following solutions (A preferred): (A) dist. water, 100 cc.; toluidine blue, 0.25 g.; orange G, 4 g.; oxalic acid, 4 g. (B) dist. water, 100 cc.; methylene blue, 0.30 g.; orange G, 4 g.; oxalic acid, 4 g. (C) dist. water, 100 cc.; malachite green, 0.20 g.; orange G, 4 g.; oxalic acid, 4 g. (D) dist. water, 100 cc.; methyl green, 0.30 g.; orange G, 4 g.; oxalic acid, 4 g. In making up the stains, heat, cool and filter. Stain 1 to 2 min. or more, blot dry and pass directly into 95% alcohol to differentiate. If necessary give a preliminary treatment with a little anilin oil in 80% alcohol. There is good differentiation of epithelium, connective tissue (including reticular tissue), cartilage, bone, striations and sarcoplasm of skeletal muscle and central and peripheral nervous tissue.—A. B. Dawson.

ELLIOTT, K. A. C. and BAKER, Z. The effects of oxidation-reduction potential indicator dyes on the metabolism of tumor and normal tissues. *Biochem. J.*, 29, 2396-2404. 1935.

This article is a study on the metabolism of thin slices of various tissues in the presence or absence of glucose as well as a number of dyes functioning as reversible oxidation-reduction systems. Dyes used were: 4 from the indophenol group (Eastman Kodak Co.); indigo carmine and two other sulfonated dyes (La Motte); Bindschedler's green and *m*-toluylene-diamine-indophenol (British Drug Houses); thionin (Coleman & Bell Co.); "prune" (Ciba and Co.); $ZnCl_2$ -free methylene blue (Merck, Darmstadt); Nile blue A and cresyl violet (National Aniline & Chemical Co.). The tissues used were: kidney, brain, retina, testis, liver and tumor from rat and rabbit. Results: The majority of the dyes in a $10^{-3}M$ concn. accelerate the respiration of tumor tissue in presence of glucose. These dyes in higher concentrations inhibit respiration of the other tissues. Thionin, "prune," methylene blue and cresyl violet in $10^{-3}M$ concentration accelerate respiration of tumor slices in glucose medium, and increase aerobic glycolysis. In $10^{-5}M$ concentration, the effects of dyes are less marked.—J. A. de Tomasi.

HAYMAKER, W. and SÁNCHEZ-PÉREZ, J. M. Rio-Hortega's double silver impregnation technique adapted to the staining of tissue cultures. *Science*, 82, 355-6. 1935.

A technic for silver staining of tissue cultures *in situ* in the plasma clot which has proven to be quick, simple and dependable has been developed as follows: After removing all paraffin and vaseline from cover slip, fix 24 hr. in a 1:1 mixture of 10% neutral formalin and normal physiol. saline. Wash for 5 min. in Petri dishes containing 30 cc. doubly dist. water, plus 6 drops NH_4OH , for thin clots on cover slips; up to 15 min. in Carrel flasks for thicker clots. Wash in dist. water. Transfer to the following: 2% $AgNO_3$, 30 cc.; 95% alcohol, 50 drops; pyridine, 25 drops; NH_4OH , 5 drops. This is heated slowly to 40° C. until a yellow color develops (about 12 min.). Wash in dist. water. Transfer to the following: Ag_2CO_3 solution, 30 cc.; 95% alcohol, 25 drops; pyridine, 15 drops. Heat slowly to 40° C. until the fragments take on a brown color (about 9 min.). Prepare the Ag_2CO_3 solution as follows: Add 20 cc. of 5% Na_2CO_3 to 5 cc. of 10% $AgNO_3$; dissolve the white precipitate by adding just enough NH_4OH , drop by drop, and make up to 75 cc. with dist. water. Wash in dist. water. Reduce in 1% formalin. Wash in dist. water. Tone in 1:500 $AuCl_3$. Heat slowly to 40° C. until a violet color develops (about 10 min.). Fix a few minutes in 1% "sodium hyposulfite". Run up thru the alcohols, xylol, balsam.—J. A. de Tomasi.

HINGST, H. E. A note on making permanent preparations of anopheline mid-guts. *Amer. J. Hyg.*, 22, 278-82. 1935.

Fix the specimen for 5 min. in the following solution: abs. alcohol, 90 parts; formalin, 7 parts; acetic acid, 3 parts. This is best done by drawing the fixing

fluid under the cover slip on one side while drawing off the dissecting medium with a strip of filter paper on the opposite side. The gut is not thoroly permeated by the fixative until it becomes milky white in color. This should take only a few seconds. Cover the entire slide with the fixative for another 5 min. Carefully lift (not slide) the cover slip with the aid of dissecting needles. The gut usually adheres to either the slide or the cover slip, otherwise subsequent handling will be extremely difficult. Flush the specimen with 70% alcohol and then cover it with the alcohol for 5 min. Replace with 50% alcohol for 5 min. Stain with Weigert's iron hematoxylin for 30 sec. (Solution No. 1: hematoxylin crystals, 1 g.; 95% alcohol, 100 cc. Solution No. 2: liq. ferri sesquichloride, 4 cc.; dist. water, 95 cc. Just prior to staining take 0.5 cc. of each solution and add to 25 cc. of 50% alcohol.) Flush with 50% alcohol. Cover with 70% alcohol for 5 min. followed by 95% alcohol for another 5 min. Counterstain with a weak solution of Bordeaux red in abs. alcohol (weak enough so that the specimen will not overstain in 30 sec.). Flush with abs. alcohol. Cover with Euparal and a cover slip.—*G. H. Chapman.*

KAY, W. W. and WHITEHEAD, R. The staining of fat with Sudan IV. *J. Path. & Bact.*, 41, 303-4. 1935.

Sudan IV (toluene-azo-toluene-azo-beta-naphthol), as prepared by the British Drug House Ltd., gives a darker stain for fats than Sudan III. The author prepares the following stock solution: 22 g. Sudan IV to one liter abs. alcohol, boiled gently until dissolved. For use, add 9 parts (by volume) of this stock solution to 7 of 45% alcohol, and filter after standing 1 hr. Use while quite fresh. The technic as applied to formol fixed frozen sections is as follows: 50% alcohol, 5 min.; Sudan IV (prepared as above) 30 min. at 37° C., turning sections once; 50% alcohol, a few seconds; dist. water, a few minutes; filtered hemalum (time unspecified); tap water, several minutes; mount in glycerin jelly.—*F. Warbritton.*

KERNOHAN, J. W. Adaptation of formalin-fixed tissue to Mallory's phosphotungstic acid hematoxylin stain. *J. Techn. Methods & Bull. Int. Assoc. Med.*, 13, 82-4. 1934.

Formalin fixation is not conducive to good results with differential staining. The following technic overcomes this difficulty: Wash the formalin fixed tissue in running water or "ammonia water" for a short time. Fix 4 days in Weigert's primary mordant ($K_2Cr_2O_7$, 5 g.; CrF_3 , 2 g.; water, 100 cc.). Fix 2 days in Weigert's secondary mordant: (copper acetate, 5 g.; CrF_3 , 2.5 g.; 36% acetic acid, 5 cc.; formol, 10 cc.; water, 100 cc.) Embed in paraffin and stain as usual with any one of Mallory's differential methods.—*J. A. de Tomasi.*

MAHDIHASSAN, S. Further studies on the symbiotes of scale insects. *Arch. Protistenkunde*, 85, 61. 1935.

For permanent preparations of the symbiotic microorganisms of scale insects proceed as follows: Brush powder off insect. Place insect on a slide in a drop of a mixture composed of equal parts of glycerin, lactic acid and water. Rupture body with a fine lancet. After exudation of sufficient symbiont-containing body fluid, remove insect skin and place a cover slip on drop. Blot edges and apply a layer of quick-drying oil varnish. After a few days, when varnish is dry and hard, repaint the edge with Canada balsam over the oil varnish. Such preparations have remained unchanged for 9 years, even when stained with cotton blue.

To show the entire microflora of a young insect body, fix the larvae in modified Carnoy's fluid (abs. alcohol, 60 cc.; chloroform, 30 cc.; glacial acetic, 5 cc.; formol, 10 cc.) for 10 min. Change gradually to water. Treat with 5% KOH in the cold until the skins are quite transparent; longer treatment injures later staining. Wash cleared skins in water, treat with dilute acetic and finally transfer to alcohol. Stain with acid fuchsin or alc. eosin. Later treat with xylol containing a trace of picric acid until the skins under the microscope are just becoming yellowish. Wash in xylol and mount in Canada balsam. The microflora stands out bright red against the pale orange of the skin. A splendid photomicrograph of an insect *in toto* shows the microflora very clearly. (Staining formulae are not given. Strength of alcohol is not stated.)—*S. H. Hutner.*

McCORD, C. P., HOLDEN, F. R. and JOHNSTON, J. **Basophilic aggregation test in the lead poisoning epidemic of 1934-1935.** *Amer. J. Pub. Health*, 25, 1089-98. 1935.

The presence of excess numbers of basophilic erythrocytes in human blood is indicative of lead poisoning. Search for these forms is best made in laked cells, after staining. A modified Manson's methylene blue yielded most consistent results, the formula being: borax, 1 g., methylene blue, 2 g., boiling dist. water, 100 cc. The borax is dissolved in the water and to this is added the methylene blue. After filtering this stain is ready for use and provides a stable, satisfactory, and uniform stain for at least 2 weeks.

Thin, even blood smears are made on slides and allowed to dry. The proper drying of these smears is important; ordinarily the optimum time lies between 1 and 3 hr. After drying, $\frac{1}{2}$ of the slide is overlaid by a strip of filter paper, and cautiously there is applied with a pipet or dropper the minimum amount of methyl alcohol required to moisten the filter paper until it clings to the slide. This is allowed to dry until the filter paper becomes loose.

The slide is then immersed in a Coplin jar, containing the modified Manson stain, for approximately 10 min. It is impossible to overstain. After staining, the slides are washed in 3 or 4 changes of dist. water. Air drying is recommended.

For counting, an oil immersion objective and a 10 \times ocular with a Whipple grid are used. The basophilic aggregations in 20 fields of the unfixed portion of the slide are expressed as a percentage of the number of red blood cells counted in the fixed portion (average of 5 fields \times 4).

Basophilic aggregations over 1-1.5%, and especially over 2%, in persons exposed to lead, suggest lead absorption.—*M. W. Jennison*.

MENKE, J. F. **The hemolytic action of photofluorescein.** *Biol. Bull.*, 68, 360-2. 1935.

A photocompound of fluorescein was made by continuous irradiation of a dilute solution of sodium fluorescein: dye, 50 g.; dist. water, 2 liters. By means of a 4 $\frac{1}{2}$ -in. reading glass, the sun's rays were focused for 300 hr. on an Erlenmeyer flask containing the solution.

By the addition of a small amount of HCl (quantity not stated) the photocompound was precipitated and, after washing with dist. water, the dye was reconverted into its sodium salt by adding enough NaOH to redissolve it. The solution was then evaporated to dryness and 1 mg. of the residue dissolved in 0.1 cc. of Locke's solution. The pH was found to be 7.85.

The red blood cells of a healthy rat were washed with Locke's solution. Alkaline buffers and a water bath of 38° C. were used in each experiment.

In 9 series of experiments photofluorescein produced hemolysis in the dark just as fluorescein does in the light. Unirradiated fluorescein had no hemolytic action in the dark. Hemolysis occurred only in the buffered controls of pH 10.0, and this was slight. It required 2 hr. in the dark for a 1:2,000 dilution of the photocompound to hemolyze 1 cc. of 1% red blood cells.

Long periods of irradiation are necessary to form appreciable quantities of photofluorescein, but shorter periods will produce hemolysis when the blood cells are in contact with the dye or when the fluorescein is previously irradiated.—*W. R. Hunt*.

MENNER, E. **Ganglienzellen-Präparate für Kurszwecke.** *Zool. Anzeiger*, 110, 200-2. 1935.

For classroom preparations of multipolar ganglion cells, the author proceeds as follows: Use fresh spinal cord of pig. With scissors remove as much of the white substance as possible. Pieces of the gray substance 1-2 cm. long are placed into 50 cc. of macerating fluid (chromic acid solution 0.05%, 5 cc.; dist. water, 45 cc.) from 2-8 days depending upon temp. The following staining mixture is said to keep well: azoacid blue B (Hoechst), 2 g.; tartar emetic, 1 g.; oxalic acid, 4 g.; dist. water, 200 cc. brought to a boil, allowed to cool and filtered after 24 hr. The tissue is transferred to a dilute stain consisting of 4 parts of the above in 96 parts of dist. water for 24 hr., is rinsed rapidly in dist. water and placed for 24 hr. in 1:3 glycerin water. It is then transferred to 1:1 glycerin water for 24 hr., and placed in pure glycerin for 2 weeks where the staining reaches its optimum. Small pieces are then transferred to a drop of glycerin on a slide and gently flattened

between the slide and cover glass. The cells appear brilliant blue with dark blue nuclei. Nissl granules show clearly, axons and dendrites are pale blue. The method is said to yield permanent results.—*J. M. Thuringer.*

MERLAND, A. *Méthode simple et rapide pour l'imprégnation des astrocytes fibrillaires.* *Bull. d'Histol. Appl.*, 12, 290-1. 1935.

This method is useful in demonstrating the astrocytes of the neuroglia fibrils, with their extensions and "trompes vasculaires". The steps of the schedule are: Fix small pieces 6 days to 1 year in the following solution: NH_4Br , 10 g.; $\text{Co}(\text{NO}_3)_2$, 2 g.; neutral formol, 15 g.; dist. water, 100 g. Wash in dist. water. Cut frozen sections and wash several times in the following: $\text{Co}(\text{NO}_3)_2$, 2 g.; neutral formol, 10 g.; dist. water, 100 g. To make sure that all NH_4Br is eliminated, test with AgNO_3 . Mordant sections in the same liquid 30-45 min. at 56°C . Wash rapidly in dist. water. Impregnate in 10% AgNO_3 at 56°C . until ochre yellow (about 30-60 min.). Reduce in the following: hydroquinone, 3 g.; formol, 10 g.; dist. water, 100 g.; anhyd. Na_2SO_3 , 0.20 g. A black precipitate in this bath indicates insufficient washing of sections; these should turn almost black. If they are too light, mordanting was insufficient. Toning may follow in 0.20% AuCl_3 , or sections may be fixed directly in 10% Na_2HSO_4 . Wash, dehydrate, mount in balsam.—*J. A. de Tomasi.*

MOSER, F. *Wurstuntersuchungen nach Brekenfeld.* *Zts. Fleisch- Milchhyg.*, 45, 304-10. 1935.

For the examination of sausage meat, not only bacteriological but also histological methods are necessary. The following histological technic was found satisfactory. Cut off from various parts of the specimen at least 3 cubes having faces 1 to 1.5 cm. Fix 24 hr. in 10% formalin, wash 1 hr. in water and cut frozen sections. Liver sausage, bologna and overried material do not make satisfactory sections. Paraffin embedding is to be preferred. Stain with a 2% aq. methylene blue solution.—*J. A. de Tomasi.*

OPPENHEIMER, J. M. *Localized vital staining of teleostean embryos.* *Science*, 82, 598. 1935.

The technic of Vogt (*Arch. Entwmech.*, 106, 542-610, 1925) is modified by employing stain-impregnated cellophane, instead of agar. The cellophane is impregnated by treating with a 1 to 5% solution of the dye. It is not a bacterial medium, does not swell, is much more rigid and easier to handle than agar. Nile blue sulfate and neutral red are both satisfactory for staining the teleostean blastoderm, either in normal or double-strength Ringer's solution. Nile blue sulfate remains definitely localized for a longer time when used in combination with neutral red. In work on *Fundulus*, best results were secured by staining the blastoderms with cellophane treated in a mixture of equal parts of 1% solutions of the 2 dyes.—*J. A. de Tomasi.*

OSGOOD, E. E. and WILHELM, M. M. *Reticulocytes.* *J. Lab. & Clin. Med.*, 19, 1129. 1934.

A simple technic was worked out for reticulocyte staining which gives uniformly good results and significantly higher counts than other methods tested. Mix in a small test tube equal parts (5 drops) of oxalated venous or capillary blood and 1% brilliant cresyl blue in 0.85% NaCl solution. Let stand 1 min., mix, and make thin smears, drying in air. These smears may be counted at any time within 24 hr., but if a permanent preparation is desired, they should be counterstained with Wright's stain by the usual technic. Count all red cells and all reticulocytes in as many adjacent fields as are necessary to give a total of 500 red cells if the count is over 5%, or 1000 cells if the count is less than 5%.—*E. Bachelis.*

PENFIELD, W. *A technique for demonstrating the perivascular nerves of the pia mater and central nervous system.* *Amer. J. Path.*, 11, 1007-10. 1935.

Wash out blood by saline perfusion. Fix with a solution of 10.5% citric acid (powder) in 20% formalin. Inject fixative or leave in for 2-3 days. Dissect out blood vessels by the aid of a dissecting microscope. Wash in 2 changes of dist. water and put into 20% aq. AgNO_3 for 2 hr. Follow by 4 changes of 20%

formalin, of 100 cc. each. Pass to ammoniacal AgNO_3 (add 28% concentrated ammonia to 20% AgNO_3 drop by drop until precipitate redissolves, then add 3 drops excess). If tissue turns black add more ammonia to control rate of staining as determined under microscope. When staining is adequate put into 20% ammonia water 1 to 2 min. Wash in dilute acetic acid (concentration not given) to counteract alkalinity. Tone with gold, fix, wash, dehydrate, clear and mount.—H. A. Davenport.

PEREIRA, C. and VAZ, Z. Ganzeinbettung von Nematoden; neue und einfache Einbettung in Balsam. *Arch. Inst. Biol. Sao Paulo*, 5, 77-80. 1934.

Various methods of preparing nematodes for microscopical examination are given. (1) For material treated with any fixative suitable for a carmine stain: hold 2-12 hrs. in Semichon's acetic carmine, differentiate 5-15 min. in 1% HCl in 70% alcohol, dehydrate 5-10 min. in abs. alcohol. (2) For material treated with acetic acid suitable for picric acid staining: hold 5-10 min. in 1:150 picric acid in 80% alcohol, dehydrate 5-10 min. in abs. alcohol. (3) For material treated with acetic acid and not to be stained: dehydrate 5-10 min. in abs. alcohol. (4) Embedding technic: Immerse the stained sections in 5% celloidin, 1 min., dehydrate 3 min. in 95% alcohol, clear 20 min. in beechwood tar creosote. Mount in syrupy Canada balsam. (Cited from *Zentbl. Bakt.*, I Abt., Ref., 119, 299. 1935.)—J. A. de Tomasi.

RIEMER, B. Some practical suggestions in the preparation of bone for histological study. *J. Techn. Methods & Bull. Int. Assoc. Med. Mus.*, 13, 72-6. 1934.

Fixation: For the best preservation of cellular structure a chrome fixative (such as Helly's fluid) is found to be most satisfactory. Formalin can be used for soft or young bone, or when decalcification with HNO_3 can be accomplished within 24 hr., thus allowing satisfactory nuclear staining.

Decalcification: Ideal decalcifier is 5% aq. HNO_3 , used at 37° C. and shaken at least twice daily. The blocks should never be thicker than 2-3 mm. and 3-4 days treatment should suffice. Nine-tenths of the decalcification takes place in the first 24 hr.

Embedding: Young or spongy bones do well in paraffin, large pieces or cortical bone of adults are better in celloidin. The latter technic is carried out as follows: 46 hr. in thin (oil-like) celloidin; 48 hr. in thick (syrupy) celloidin. Transfer pieces to a large dry Petri dish and pour over a thick solution of the same celloidin. Air bubbles disappear later during hardening, which occurs in 48-72 hr., yielding a material of even consistency thruout. When this reaches a rubbery consistency, cut out in blocks, trim, and soak 24 hr. in 80% alcohol.

Spreading: Paraffin sections tend to loosen from the slide. After decerating, pass thru abs. alcohol and dip into a very thin celloidin solution. Wipe off excess liquid from back and edges of slide and transfer to 30% alcohol until ready for staining. Celloidin may be removed with alcohol-ether or by clearing 2 or more hr. in clove oil.

Staining: Celloidin sections are better stained loose by transferring with a spatula to a hematein solution. Mayer's hematein (hemalum) is most satisfactory. Ripen this for a week and stain for 6-12 hr. Overstaining, shown by uniform blue color, can be corrected with acid alcohol, following carefully the decolorization of the cement line structures. Wash 3 hr. in running tap water. Stain 10 min. in 1% aq. eosin, transfer to water, 80% alcohol, and 2 changes of 95% alcohol. Fish sections onto albuminated slides, press between lens paper, transfer for 3 hr. to clove oil. Pass into abs. alcohol for a few minutes, then xylol and mount.—J. A. de Tomasi

RULON, O. Differential reduction of Janus green during development of the chick. *Protoplasma*, 24, 346-64. 1935-36.

Chick embryos at various ages were removed from the egg and stained for about 8 minutes with Janus green in a 1/50,000 concentration in modified Locke's solution (0.9% NaCl , 0.042% KCl , 0.024% CaCl_2). They were then washed and sealed under a cover slip in the salt solution, care being taken to eliminate all air bubbles from the sealed chamber. The rate, intensity on regions of reduction of the dye were noted, the reduction changing the color from light blue to red. The yolk digesting cells in the area opaca were the most active in re-

duction. Different regions showed active reduction at different ages, the differentials in reduction corresponding closely to disintegration gradients of Hyman (1927) and Hinrichs (1927).—*Author's Abstract.*

STAFFORD, E. S. An eosin-methylene-blue technique for rapid tissue diagnosis. *Bull. Johns Hopkins Hosp.*, 55, 229. 1934.

A new technic has been worked out and found to be satisfactory for rapid tissue diagnosis. Cut small thin blocks (not over 3 mm. thick). Heat to boiling in fixing solution (15% formalin, 75 cc., abs. alcohol, 25 cc.). Freeze and cut sections after washing blocks in water. Stain 1 sec. in eosin solution (eosin, 1 g., potassium bichromate, 1 g., water, 100 cc.). Wash in water. Stain deeply in methylene blue solution (Goodpasture) 10–20 sec. (methylene blue, 1 g., potassium carbonate, 1 g., water, 400 cc.; boil 30 min., add 3 cc. glacial acetic acid, shake until precipitate dissolves and concentrate to a volume of 200 cc.). Wash in water. Mount on slide. Blot (moisten blotting paper with drop of dehydrating fluid). Dehydrate with 15–20 drops of the following solution: abs. alcohol, 1 part, acetone, 5 parts. Dip slide into xylol. Mount in balsam.

The coloring and appearance of sections are similar to those of hematoxylin-eosin.—*E. Bachelis*

SZEPSENWOL, J. Un procédé d'imprégnation argentique applicable aux jaunes larves d'amphibiens. *Compt. Rend. Soc. Biol.*, 116, 1280–1. 1934.

Silver impregnation of young amphibian larvae has heretofore proved a difficult task even after lengthy fixation in 10–15% formalin. The author's method is: Fix material for at least 2–3 months, or better for a year, in 10–15% formalin or for 30 days in 15% formalin followed by 95% alcohol 10–15 days. Wash in running water for 24 hr. Pyridine, 10 days. Dist. water 24 hr. Place in 3% silver nitrate, 3–4 weeks in the dark at 28–30°. The larvae should appear dark brown with a reddish tinge. Wash rapidly in dist. water. Treat in 10% ammoniacal silver nitrate solution 6–8 hr. Wash rapidly in dist. water, and transfer to neutral 20% formalin. In successful impregnations, the larvae will retain their brown color, the formalin remaining clear. Otherwise return larvae for a few days into 3% silver nitrate, followed by the remainder of the treatment.—*J. M. Thuringer.*

TAMURA, O. Ueber eigenartige Riesensternzellen von Urodelen mit nur supravital färbbaren Granulis. *Folia. Anat. Jap.*, 13, 573–6. 1935.

A new type of cell is described which is found in the peritoneum of certain amphibia (the Urodela). With needle and forceps the light membrane is separated from the belly wall as a pellicle which can be stretched out and secured to a cover glass. Immersed in water, the tissue shows star-like cell formations of a brown-yellowish color and beautifully ramified. Their bulk is made up of numerous fine bright granules. Any fixing fluid will cause the contour of cell and granules to disappear; the latter can only be revealed by supravital staining with certain dyes. Stain not longer than 30–60 sec. with methylene blue, toluidine blue, neutral red or Nile blue sulfate; rinse in water and examine in water immersion. The stained preparation can subsequently be fixed with formol or sublimate, rinsed, dried and mounted in balsam. Hematoxylin and eosin used on fixed material stain only the nucleus. As a differential characteristic it is found that polychrome methylene blue stains the granules of mast cells red violet and those of the giant star cells, blue.—*J. A. de Tomasi.*

WALLART, J. Une modification de la méthode argentique de Bielschowsky, pour organes riches en lipoides. *Bull. d'Histol. Appl.*, 12, 254–6. 1935.

The observation that lipids and other fatty bodies in the ovary hinder the penetration of silver solutions, suggested the following technic: Fix at least 2 weeks in dilutions of neutral formol in dist. water 1:9, 1:4, or 1:2. Blocks of 3–5 mm. thickness are fixed again, using the following liquid: Equal parts of pyridine, ether, and abs. alcohol, 5 cc., 4% aq. NaOH 1 drop. Wash 12–24 hr. in running water, and for the same time in dist. water, changing every 2 hr. Impregnate 5 days at 36–37° C. in 1.5 to 3% AgNO₃. Rinse a few seconds in dist. water. Soak 24–36 hr. at room temp. in the following: 10% AgNO₃, 10 cc.; 40% NaOH, 5 drops; redissolve the precipitate by stirring with addition of NH₄OH, drop by drop, until only a few black specks are left; make up to 100 cc.

with dist. water. Wash 2 hr. in dist. water, changing every 30 min. Reduce 12-18 hr. in neutral formol diluted 1:9 with dist. water. Dehydrate rapidly with abs. alcohol, followed by toluene, then paraffin (this should take from 10-12 hr.). Cut 7-10 μ sections and treat with the gold solution. Best results were obtained on cat and sow ovaries.—J. A. de Tomasi.

WILDER, H. C. An improved technique for silver impregnation of reticulum fibers. *Amer. J. Path.*, 11, 817-20. 1935.

Fix in 10% formalin, acetic-Zenker or formol-Zenker. Paraffin, celloidin, or frozen sections. Put material into 0.25% KMnO_4 or 10% phosphomolybdic acid for 1 min. Rinse in dist. water, transfer to a solution of concentrated HBr (Merck 34%), 1 part; dist. water, 3 parts, for 1 min. This can be omitted after treatment with phosphomolybdic acid. Wash in tap water and dip in 1% uranium nitrate (Na free) for 5 sec. or less. Wash in dist. water 10-20 sec. and impregnate in Foot's silver diammino hydroxide (Amer. J. Path., 5, 223-38, 1929) for 1 min. Dip quickly in 95% alcohol and reduce 1 min. in the following solution: dist. water, 50 cc.; 40% neutral (MgCO_3) formalin, 0.5 cc.; 1% uranium nitrate, 1.5 cc. Wash, tone with gold, fix, wash, counterstain if desired, dehydrate, and mount.—H. A. Davenport.

PLANT MICROTECHNIC

DUFRENOY, J. A method for embedding plant tissues without dehydration. *Science*, 82, 335-6. 1935.

Methylal, $\text{CH}_3(\text{OCH}_3)_2$, is a solvent for lipoids and dissolves in water. It is, therefore, a good intermediate between water and fats, and can be used to advantage in carrying plant tissues from killing fluid to paraffin. After fixation, wash in water and transfer thru the following series into paraffin, holding the material 1 hr. at each step: 1:1 methylal-water; methylal; methylal dehydrated and neutralized with anhyd. Na_2CO_3 ; 1:1 methylal-paraffin oil. Warm tissue on a water bath and transfer to paraffin of low melting point. Within an hour, transfer to grade of paraffin required for embedding. The finer details of structure are well preserved, the lignified tissues retain a soft waxy texture.—J. A. de Tomasi.

KOSTOFF, D. and KENDALL, J. Irregular meiosis in *Lycium Halimifolium* Mill., produced by Gall Mites (Eriophyes). *J. Genetics*, 21, 113-5. 1929.

Description of phenomena observed in flower buds of the host, *Lycium*, attacked by mites before and during the occurrence of meiosis.

The material was studied both in aceto-carmin and in permanent preparations. For permanent preparations, the tissues were fixed in Bouin's solution as developed by Allen, and in a copper-Bouin solution (100 cc. Allen's modification of Bouin's solution plus 3 g. of copper oxide). The former was used to study the chromosomes and the latter to study the tissues. The preparations were stained by Heidenhain's iron-hematoxylin and orange G.—R. W. Cumley.

LAWRENCE, W. J. C. The genetics and cytology of *Dahlia* species. *J. Genetics*, 21, 125-59. 1929.

The following species were examined: *D. variabilis*, *D. Merckii*, *D. Maroni*, *D. coccinea*, *D. coronata*, *D. imperialis*, *Bidens atrosanguinea*, *Hidalgoa Wercklei*. Good pollen mother-cell divisions were not obtained from *D. Maroni*, *imperialis*, or *coronata*. Apparently, the other species furnished good material.

The somatic counts were made from root tips. *D. variabilis* and *D. coronata* were fixed in Newton's modification of Flemming's fluid. The other species were fixed in a solution of 1% chromic acid, 60 cc.; 2% osmic acid, 20 cc.; 5% glacial acetic acid, 25 cc., which gave better results. Various fixatives were used for the pollen mother-cell material, the best results being obtained by Kihara's method (Carnoy 1 min., followed by Flemming).

By trimming off the outer bracts and quartering the capitulum the florets can be cut *en bloc* transversely or longitudinally. Quite good fixations were obtained by this method altho the fixing of individual florets gave more consistent results. Smear preparations were tried without success.

Embedded material of root tips was cut 10 μ thick, and anthers 14 μ . The sections were stained by Newton's gentian violet method.—R. W. Cumley.

NEWTON, W. C. F. and DARLINGTON, C. D. Meiosis in polyploids. Part I. Triploid and pentaploid tulips. *J. Genetics*, 21, 1-15. 1929.

Material was fixed in Flemming's solution of the composition: 1% chromic acid, 60 cc.; 2% osmic acid, 20 cc.; 10% acetic acid, 25 cc.

Newton's method of gentian violet staining was used thruout. This consists of staining 3-10 min. in 1% supersaturated aq. solution of Grubler's gentian violet, rinsing in water rapidly, and then for 15-60 sec. in a solution of 1% iodine and 1% KI in 80% alcohol, differentiating rapidly in 2 washes of abs. alcohol and 2 washes of clove oil, and mounting from xylol. The method was of particular value in studying side views of metaphase where large multiple chromosome associations were formed.

In the tulips where smears are to be made of the anthers, it is possible to cut across the 4 loculi at one end of the anther and squeeze out the pollen mother-cells, which may then be smeared by drawing a second slide across the first. Fixations of the smears with osmic vapour did not give satisfactory results.

Tulips provide unusually good material for the study of the pollen mother-cell division. The nuclei are extraordinarily resistant to the damaging effects of fixation, and synizesis is not found in smears.—*R. W. Cumley.*

PERCIVAL, J. Cytological studies of some hybrids of *Aegilops* Sp. + *W* heats, and of some hybrids between different species of *Aegilops*. *J. Genetics*, 22, 201-78. 1930.

Cytological investigations were made of the meiotic phase in the pollen mother-cells of all the hybrids. Carnoy's solution was employed for permanent preparations and gave exceedingly good results, even in the very fine spireme stage of early prophase. It was allowed to act for not less than 30 min., followed by careful washing with abs. alcohol to remove all traces of acetic acid.

Longitudinal and transverse sections were cut 12-14 μ thick, and stained with Heidenhain's iron-alum-hematoxylin.—*R. W. Cumley.*

STRUGGER, S. Beiträge zur Gewebephysiologie der Wurzel. Zur Analyse und Methodik der Vitalfärbung pflanzlicher Zellen mit Neutralrot. *Protoplasma*, 24, 108-27. 1935.

Vital staining of *Trianea* root hairs with neutral red in aq. solution at a pH more acid than 6.4 is restricted to the cell wall. In more alkaline solutions the cell wall decolorizes and the stain accumulates in the vacuolar sap.—*R. Chambers.*

MICROÖRGANISMS

DELÉTANG, R. Contribution à l'étude de la réaction de Gram. *Les Presses Modernes, Paris*. 134 pp. 1933.

Part I is a critical study of the Gram stain technic and of the physical and chemical theories that attempt to explain it. Several methods are discussed at length, with their specific advantages and limitations. The role of microorganisms is also analyzed with respect to conditions of the culture and site of the reaction within the individual cells. Part II deals with the author's research on successive steps of the staining procedure and of the factors influencing it. These include a study of the significance of cellular lipids, nucleoproteids and lipoproteids and resistance of the cell to decoloration. The Gram reaction is attributed to the slight permeability of Gram-positive cells to alc. iodine and also to the adsorption of dye on the surface of the cytoplasmic micellae (especially those of nucleo-protein nature). It is brought about by a combination of the Gram complex with certain lipids, particularly lipoproteids, with unsaturated fatty acids. The site of the reaction is thought to be the cytoplasm rather than the membrane. The differentiation between bacteria suggests that distinct physico-chemical differences exist between Gram-positive and Gram-negative organisms. The Gram stain should be regarded, not only as a tinctorial reaction, but also as an aid to bacterial classification.—*J. A. de Tomasi.*

GIEMSA. Ueber eine bemerkenswerte Fehlerquelle bei der färberischen Darstellung der Schüffner-Tüpfelung. *Münch. med. Wschr.*, 1975-6. 1935.

With tertian malaria it is found that the staining of the Schüffner granula (Schüffner stippling) is missing because the fixation has been performed with an

insufficiently purified brand of methyl alcohol. Two grades of methanol are produced by the Merck Co.; one labeled *purissimus* (acetone-free) has proven to be satisfactory, the other called *purus* has not. (Cited from Zentbl. Bakt., I Ref., 120, 174. 1926.)—J. A. de Tomasi.

HAGELSTEIN, R. **The Mycetozoa.** N. Y. Micro. Soc. Bull., 1, Feb., 1936.

The microscopical examination of these organisms, otherwise called Myxomycetes or slime molds, should first be made as an opaque object, with a few of the sporangia blown out by a small hand blower to free them from spores. Then these sporangia are picked off, placed on a slide with water under a cover glass and examined by transmitted light. If the air cannot thus be driven out, a mixture of water and alcohol will assist. Badly contracted spores may be swollen rapidly by the admission of a drop of 5% aq. KOH, altho this tends to alter the color of the spores of certain species. Permanent preparations may be mounted in glycerin, glycerin jelly, or Canada balsam.—J. A. de Tomasi.

HALBERT, V. A. **A study in the differentiation of Escherichia coli and Aerobacter aerogenes by the bacteriostatic action of organic dyes.** J. Bact., 30, 653-4. 1935.

To a basic medium of standard extract agar (pH 7.8) a series of water soluble dyes are added in the following concentrations: 1:500; 1:1,000; 1:3,333; 1:5,000; 1:10,000. One-half of each plate is poured with plain agar, the other half with the agar and dye. The plates are streaked with the cultures and read after 24 hr. incubation. Dahlia B, Janus green B, martius yellow, benzyl violet, and aldehyde green (source not given) favored the growth of *Escherichia coli*, but inhibited *Aerobacter aerogenes*.—J. A. de Tomasi.

KABELIK, J. **Eine Modifikation der Dold'schen Färbung des B. diphtheriae.** Prakt. lek., p. 207. 1935.

A modification of Dold's stain (Zentbl. Bakt., I Abt., Orig., 124, 220, 1932; *abs.* Stain Techn., 8, 40) for *B. diphtheriae* consists in the substitution of brilliant green for anilin green. It has proven of value in many cases of diphtheria and pseudodiphtheria. This stain, developed along the lines of the Gram stain, gives better tinting (green) of the diphtheria organism than of the pseudodiphtheria organisms (brown). If this method is supplemented by culture on Löffler's blood serum, the specific organisms can be easily detected and isolated in 24-48 hr. (Cited from Zentbl. Bakt., I Abt., Ref., 120, 4. 1935.)—J. A. de Tomasi.

KOCJAN, L. **Einige Resultate mit der Färbung der Milzbrandbazillen nach der modifizierten Foth'schen Methode.** Jug. Veterinarski Glasnik, No. 6, 1935.

In 5 cases out of 35 diagnoses of anthrax based upon cultural, staining, and biochemical characteristics, van Heelsbergen's modified Foth's stain proved far superior to all other methods. (Original article not seen, and no details of technic are given in abstract above cited.) (Cited from Zentbl. Bakt., I Abt., Ref., 119, 385. 1935.)—J. A. de Tomasi.

KRAJIAN, A. A. **A rapid method of staining Spirochaeta pallida in single sections of tissue.** Arch. Dermat. & Syphil., 32, 764-7. 1935.

The following technic is suggested: Fix fresh blocks of tissue about 5 mm. thick in 10% formalin heated to 67° C. for 10 min. (not required for formaldehyde fixed tissues). Cut sections 5 to 10 μ on a freezing microtome. Place for 5 min. in a fresh 1% aq. solution of sodium cobalti-nitrite. Wash in two changes of dist. water. Place for 15 min. in the following solution previously heated and held at 67° C.: uranium nitrate, 1 g.; 85% formic acid C. P., 3 cc.; glycerin C. P., 5 cc.; acetone, 10 cc.; 95% alcohol, 10 cc. Wash in two changes of dist. water. Put in 0.75% aq. AgNO₃ 1 hr. at 67° C. Rinse rapidly in dist. water. Develop 3 min. in 2 cc. of the following solution: Hydroquinone, 0.31 g.; sodium sulfite, 0.06 g.; acetone, 2.5 cc.; neutral C. P. formalin, 2.5 cc.; pyridine, 2.5 cc.; satd. solution of mastic in 95% alcohol, 2.5 cc.; dist. water, 15.0 cc. Expose to light 15 min. before use. The solution keeps from 2 to 3 weeks in a dark cool place. Prior to use add 1 drop Mayer's fixative. While developing, work under a 60

watt lamp 4 ft. away. Wash a few seconds in dist. water. Return to the 0.75% AgNO_3 for 15 to 25 sec. Wash in 2 changes of dist. water. Draw onto slide and blot. Dehydrate in abs. alcohol 2 min. Clear in xylol 2 min. Mount in dammar resin. A control containing spirochaetes should be run simultaneously. The method can also be used for bacteria.—G. H. Chapman.

RIPPEL, H. Fossil microorganisms in a permian salt deposit. *Arch. Mikrob.*, 6, 350-8. 1935.

The presence of bacteria in salt can be shown by the direct soil staining technic of Conn, Winogradsky and Rossi-Cholodny, using carbol-erythrosin (phenol 5%, erythrosin 2%, in water). The sample is washed, then dissolved in sterile distilled water, and centrifuged. The sediment is dehydrated with 96% alcohol, again centrifuged, and then shaken with ether. The treatment with ether is necessary to remove minute oil droplets which take up the stain and may be confused with coccoid forms. The clear ether is decanted, a small amount of water added and heated to drive off the remaining ether. After usual drying and fixing in the flame, the sediment on the slide is stained for 24 hr. in carbol-erythrosin and washed with water.—P. Esau.

SEIFRIZ, W. and ZETZMANN, M. A slime mold pigment as indicator of acidity. *Protoplasma*, 23, 175-9. 1935.

The yellow pigment of the slime mold, *Physarum polycephalum*, is an acid-alkaline indicator with a color range from deep red-orange to bright yellow-green. The pigment is dissolved out by shaking the organism in water. The colors are as follows: pH < 1.0, deep chrome; pH 1.6, deep orange; pH 3, golden yellow; pH 5.0, dark yellow; pH 6.2, light yellow; pH 7.0, sulfur; pH 8.0 green-yellow; pH > 8.0, yellow-green.—R. Chambers.

STONE, W. S. A method of eliminating *Blastocystis hominis* from cultures of *Entamoeba histolytica*. *J. Lab. & Clin. Med.*, 21, 190-1. 1935.

Growth and reproduction of *E. histolytica* are occasionally impeded by the presence of the flagellate-like fungus *B. hominis*. The influence of various concentrations of neutral acriflavine was studied, leading to the following technic: Add to each of the mixed cultures 0.05 cc. of a dry sterile mixture of 2 parts powdered rice starch and 1 part powdered animal charcoal. Add enough 1:10,000 neutral acriflavine in Ringer's solution to the supernatant fluid of the culture on Boeck-Drbohlav medium to make a 1:50,000 solution. Incubate 24 hr. at 37° C. Transfer by repeating the same procedure. Prepare a sterile mixture of 1 part horse serum and 7 parts of the following modified Locke's solution: NaCl, 9.0 g.; CaCl_2 , 0.24 g.; KCl, 0.42 g.; NaHCO_3 , 0.2 g.; N lactic acid, 0.23 cc.; dist. water, 1,000 cc. Add 5 cc. of this mixture to egg slants and transfer the culture a few times. A final growth on Boeck-Drbohlav alone yields pure cultures of the amoebae.—J. A. de Tomasi.

ZEETTI, R. Nouvelle méthode pour colorer facilement les spores des schizomycètes. *Boll. Sez. Ital. Soc. Int. Micro.*, 7, 310-1. 1935.

This new spore stain claims the advantage of eliminating the precarious step of decolorization usually resorted to for the purpose of differentiating between spores and vegetative forms. Proceed as follows: Fix the preparations by flaming. Flood with the following solution: Lugol's iodine, 100 cc.; eosin Y, 5 g.; phenol crystals, 5 g.; filter thru paper before storing. Heat for 5 min. (boil 2-3 times); boiling will cause no precipitation. Wash quickly in dist. water. Counterstain $1\frac{1}{2}$ -2 min. in sat. alc. methylene blue diluted 1:20 with dist. water. Rinse, air dry and mount. Vegetative forms stain blue, spores a deep pink.—J. A. de Tomasi.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the January number of this journal.

STAINS CERTIFIED DECEMBER 1, 1935 TO FEBRUARY 29, 1936*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Methylene blue	CA 15	86%	For histology, bacteriology, and as constituent of blood stains	Dec. 11, 1935
Cresyl violet	NW 5	97%	For use in histology	Dec. 13, 1935
Brilliant green	NBg 1	95%	For use in bacteriological media	Dec. 16, 1935
Crystal violet	DC 1	92%	For use in bacteriological media	Dec. 16, 1935
Gentian violet	CB 12	81%	For use in histology, bacteriology and cytology	Dec. 19, 1935
Methylene violet	NLv 3	—	As constituent of blood stains	Jan. 28, 1936
Orange G	NO 6	87%	As counterstain in histology	Feb. 6, 1936
Methylene blue	LA 6	84%	For histology, bacteriology, and as constituent of blood stains	Feb. 8, 1936
Carmine	NCa 5	—	For use in histology and cytology	Feb. 27, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

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A RAPID CELLOIDIN METHOD FOR THE ROTARY MICROTOME

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ABSTRACT.—A method is described which combines the writer's hot celloidin technic¹ with a form of the clearing-before-cutting procedure. The method requires only 16–17 days and yields a block which may be cut in any microtome, the sections being as thin as those afforded by paraffin with comparable material. The advantages of celloidin over paraffin, listed in the writer's earlier paper, are retained in the present method which, altho consuming more time than the hot process, requires less skill and gives superior results.

In an earlier paper,¹ which must be consulted by the reader of the present one, the writer described a rapid celloidin method, the central feature of which is the elimination of evaporation as a means of increasing the concentration of the celloidin during infiltration and inclusion. In the classical cold celloidin method, evaporation of the dual solvent is permitted to start early in the procedure and, as the ether escapes faster than the alcohol, the result is a matrix which apparently "sets" largely by precipitation from a no longer adequate solvent, yielding a block whose actual celloidin content is low, and which is too milky and rubbery to permit easy orientation and thin sectioning.

The "hot" method involves a forced increase in concentration by frequent changes of the solution, and under its conditions the concentration can be carried much higher before solidification is permitted. While the hot method is very rapid, it has two disadvantages: a sliding microtome must be used, and considerable skill is needed in the cutting process if the most is to be gained from the method in the way of thin, perfect sections.

The technic here described combines the rapid infiltration of the hot process with a different method of inclusion, in which carefully controlled evaporation and impregnation with cedar oil are employed.

¹Walls, G. L. The hot celloidin technic for animal tissues. *Stain Techn.*, 7, 135–145. 1932.

The latter step will be familiar, as it is the essential departure of the clearing-before-cutting modification of the ordinary cold method. It permits dry storage and dry cutting in a rotary microtome—which should make the present method appeal to those who have only a rotary machine available and wish to be able to secure their occasional celloidin blocks in a reasonable time. The writer would mention, however, that he personally employs this method not as an occasional last-resort substitute for paraffin, but routinely in decided preference to the latter matrix for any sort of material.

PROCEDURE

Secure the materials, and prepare the solutions listed on pp. 136–138 of the writer's earlier paper, substituting 15% celloidin for the 14% solution and omitting the 50–50 mixture of alcohol and glycerin. In place of the latter, some Gilson's mixture (50–50 chloroform and cedar oil) and some cedar oil will be needed. The latter should be the kind marked "water-white" or "microscopic, for clearing"—*not* the thick kind used for immersion optics. A few short style $\frac{1}{2}$ -pint clamp-top Mason jars (or similar fruit-preserving jars), with a 100 cc. beaker for each, will be required.

Follow the hot process, using 2%, 4%, 6%, 10%, and 15% solutions for 24 hours each²; but add no dry celloidin with the thickest solution as in the straight hot method. After 24 hours in 15% celloidin in the incubator, transfer the pieces of tissue to about 60 cc. of 15% in a beaker, place the latter in one of the Mason jars, and clamp the top on the jar *leaving off the rubber sealing-ring*.

Slow evaporation will now begin, and in about four days the mass will be deeply concave on top. Run a dissecting needle around the top of the mass to free it from the glass, and replace the top on the jar (if this results in the formation of bubbles at the bottom of the beaker, wait longer the next time). Weather conditions will govern the duration of the inclusion process, but after a total of five to eight days in the beaker the mass should be capable of being shaken out into the hand. It may be necessary to pull the mass out with forceps and replace it a day or so before this stage is reached in order that it will free itself naturally from the beaker and not remain stuck to the latter until it has become *too* hard.

If the mass feels dry, is fairly hard to squeeze, and does not show light fingerprints readily, it is ready to block: trim (not too closely; dry all trimmings, to re-dissolve for later use) and cement to a fiber

²It is best to invert the embedding bottles in the incubator to seal the corks well. This point was inadvertently omitted from the description of the hot method.

microtome block³ with 15% celloidin, first dipping the surfaces to be apposed in ether-alcohol. Place in Gilson's mixture for 24 hours, then blot the block and allow it to stand in air for ten to twenty minutes, depending upon size. Transfer to cedar oil. Blocks must remain here for at least three days before cutting, and may be stored indefinitely in cedar oil either before or after sections have been cut from them. After a month or so they may even be stored dry in an air-tight jar.

To section, wipe the block dry and cut in either a rotary or a heavy sliding microtome, with the knife in standard paraffin position—not slanted as for wet celloidin blocks. Cut rapidly and transfer sections in batches of ten or more, with brush or forceps, to 80% alcohol.

Rinse the sections in 95% alcohol to remove the cedar oil, and transfer to clean 80%. They can stay here indefinitely. They will, in fact, be toughened somewhat by the 80% in a week or so.

When ready to stain, transfer directly to water (adding a *trace* of soap to the water if the "scooting-about" phenomenon promises damage to very delicate sections) and follow the procedure outlined on pp. 141-142 of the paper on the hot process.

REMARKS

This "hot-dry" method is not entirely foolproof, as some judgment is required in determining the stopping point of the evaporation. The use of the fruit jar and beaker, however, slows the latter process to such a degree that both of the solvent liquids are able to escape properly, so that the matrix remains transparent and is high in final celloidin content. The finished block is very hard and can usually be cut at 6μ , often thinner.

The evaporation can be stopped at any time, if one has not time to block the matrix when it is ready, simply by putting the sealing-ring in place on the clamped jar.

There are two difficulties which may arise:

- 1) When flat pieces of tissue, such as patches of skin, etc., are to be embedded, they must not be allowed to lie on the bottom of the beaker, else there will be no celloidin to speak of on one side of them when blocked. The writer has tried various metal props, which were pulled out of the mass at the time of blocking, but has discarded them in favor of the following procedures, between which there is little to choose—one involving a little more trouble, the other a little more time: *a*) When the mass is at blocking consistency, trim the concave top to a plane with a knife, invert the mass in the beaker,

³Which is labeled with India ink on its under side.

and add a little more 15% celloidin. Replace in the Mason jar, and in a day or two the mass will regain homogeneity. There will now be a safe thickness of celloidin on the formerly exposed side of the tissue. b) When doing routine blocking, cut discs from the mass before trimming out the cylinder containing the tissue and store these in an air-tight jar. Place one of the discs in the bottom of the beaker whenever starting a flat piece of tissue thru the evaporation process.

2) Bubbles may form in the beaker (especially in warm weather) and, if large and in contact with the tissue, may give difficulty in sectioning. Bubble formation is an indication that evaporation is taking place too rapidly, or else that the mass has been freed from the sides of the beaker too soon. If the bubbles appear while the celloidin is still quite fluid, they may be caused to shrink and vanish by putting the sealing-ring on the Mason jar and placing the latter in a refrigerator for a few hours (allowing the jar to regain room temperature before opening it and removing the sealing-ring). If they form after the mass has become semi-solid, the only remedy is to fill those which are in dangerous locations at the time of blocking with 15% celloidin using a hypodermic syringe.

MICROTOME KNIFE SHARPENERS OPERATING ON THE ABRASIVE-GROUND GLASS PRINCIPLE¹

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ABSTRACT.—Developments in the abrasive-ground glass type of microtome knife sharpener have been traced from 1857 to the present. Merits and disadvantages of each apparatus are critically discussed, particularly of the two practical power sharpeners, namely, the horizontal revolving disc and the vertical wheel. A new vertical wheel model, designed to facilitate the sharpening of long, sliding microtome knives, either with a chisel-shaped or a symmetrical bevel, is described. Notable mechanical features include: shaft rotating on V-slots as sole adjustable part, thereby assuring accuracy of alignment; steel guide bar 45.7 cm. long; glass wheel mounted on its own axis, separate from motor; simple and sturdy construction with single casting constituting entire framework.

HISTORICAL

Altho second in importance only to the microscope itself, the earliest "microtome" was simply a knife or razor. Yet so proficient were early microscopists in its use that as late as fifty years ago the finest and most critical sections were cut free hand. Little wonder, then, that the condition of the knife was a matter for primary consideration and that no pains were spared in order to produce a perfect cutting edge. That they succeeded to a remarkable degree is shown by the fact that the best sharpening methods known today are fundamentally identical with those of eighty years ago. It seems proper, therefore, to preface a discussion of modern microtome knife sharpeners with a sketch of their predecessors.

Realizing that it was rarely possible to have microtome knives well sharpened by another, Hugo von Mohl (1857) developed a technic of his own, which, he predicted, anyone with a little mechanical skill could soon acquire. His principal innovation was the use of a thick plate glass having a plane and very finely ground surface, upon which the previously honed knife was polished. After adding to the plate a creamy paste of a fine abrasive powder, usually Vienna chalk, a circular motion was imparted to the knife, it being considered unessential for polishing that the knife always move against its edge as in honing. The blade was turned over at frequent intervals and progress

¹Apparatus constructed for an investigation supported by a grant from The Rockefeller Foundation to Prof. T. H. Goodspeed.

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was ascertained by repeated inspections under the microscope. This method was apparently widely adopted and was favored by Harting (1866), Dippel (1885), Moll (1892), Bouin and others. One reason for this popularity, mentioned by Harting (1866), was that good hones were expensive and soon became concave, whereas one glass plate could be readily discarded for another. It thus simplified the problem of maintaining a knife with a straight edge and a straight bevel. Twenty-seven years after its introduction, Dippel (1885) wrote, "For polishing, the method recommended by Hugo von Mohl is unsurpassed."

Naturally this initial success led to attempts to improve upon both the form and usefulness of the original apparatus. The glass soon came to be used as a base for the coarser grinding as well as for the polishing, and later Lendvai (1909) advocated a set of three plates, one for each grade of abrasive powder. The knives were held either flat on the plate or against tubular steel backs with which they were equipped, but the glass plates were still similar in size to the regular hones and continued to be used in the same manner. In a recent apparatus designated as the "Schmid Sharpener" (A. H. Thomas Co., Philadelphia) the knife holder consists of a carriage mounted on wheels and a rod which screws into the back of the knife. A smooth glass plate serves as a runway for the holder while the knife is alternately pushed and pulled across a ground glass surface large enough (35 x 35 cm.) to accommodate its full length. Altho this constituted a definite advance, the sharpening process still remained a task both laborious and time consuming.

The application of power to the problem of microtome knife sharpening has resulted in the development of three types of apparatus. For brevity, these may be referred to as the (1) sliding plate, (2) horizontal revolving disc, and (3) vertical wheel types. The sliding plate mechanism was built by Funck (1910), who first used water power and later an electric motor. Sliding back and forth three times a second on a 14 cm. track, the glass plate had an average linear velocity of 50 meters per minute. The knife was held against the plate by hand, either perpendicularly or obliquely to the line of motion, during both forward and backward strokes. Various grades of levigated alumina were employed to form abrasive pastes. Inherently impractical for a sharpener from the mechanical standpoint, it is improbable that this type of construction will ever be widely used. Funck himself seems not to have been satisfied with it, for he also designed a model with a horizontal revolving disc (200 r. p. m.). He did not regard the higher speed of the latter as an advantage, however, and was deterred from executing his plans

by the disadvantage of rapid loss of abrasive, possible accidental injury, and the unequal grinding of the knife along its length. Only the last-mentioned disadvantage possesses fundamental significance.

A contemporary, Stefan von Apathy (1913), who has recorded his dissatisfaction with knives sharpened by Funck, was using a horizontal disc at about the same time, but has supplied no further details. An apparatus of this type developed by Nageotte (1926) had a disc 43 cm. in diameter with a 20 cm. hole at the center, thus furnishing an effective surface 11.5 cm. wide. It revolved once a second (60 r. p. m.). Nageotte found a principal difficulty in constructing satisfactory knife holders and his final choice, one which fitted over the back of the knife and rested on the glass disc during sharpening, was not a desirable solution of the problem. Besides being ground away as rapidly as the knife edge, the holder required the polishing to be done at the same angle as the grinding.

For both the sliding plate and horizontal disc sharpeners, a mechanical method of supporting the knife seems best suited. Fanz (1929) has added this improvement to his automatic horizontal plate sharpener, in which the knife slowly oscillates back and forth, and periodically reverses from one side to the other. This machine is offered commercially in two sizes, 45 cm. and 75 cm. disc, with the automatic features optional. It operates at a speed of 42 revolutions per minute. Since the tangential velocity of a point on the disc increases as its distance from the center, it follows that the abrasive action on a knife is a linear function of its radial position. Simultaneously, two problems are created. The first is how to compensate for the unequal grinding of the knife along its length in order to maintain a straight edge parallel to the back. This can be done by reversing the direction of rotation of the disc during half of the sharpening process, but this may prove very difficult to do with automatic machines. It may not be necessary for rotary microtome knives. The second and more serious problem is how to maintain the ground glass surface of the disc plane. If facilities are not available for resurfacing the disc, probably the simplest and most economical, altho but partial, solution is to replace it occasionally with a new one.

VERTICAL WHEEL SHARPENERS

The vertical wheel microtome knife sharpener designed by Prof. J. A. Long (1927) of this institution, departs radically from the flat plate characteristics of all previous apparatus based on the abrasive-ground glass principle. In this case, the periphery of a disc rotating in a vertical plane forms the grinding surface. The knife edge is passed laterally across this surface by an operator who holds the

back of the knife firmly against the two sides of an L-shaped guide bar (Fig. 1). Since no auxiliary back or holder is required, a possible source of inaccuracy is eliminated and the mechanical stability increased. It is only essential that the edges of the back of the knife be straight and parallel to the cutting edge. In case the narrow (1.3 cm.) wheel should wear smooth or become uneven, it can be simply redressed. The wheel housing acts as a reservoir for the abrasive suspension, which the wheel itself automatically circulates. The concavity produced in a narrow bevel by polishing on a 15 cm.

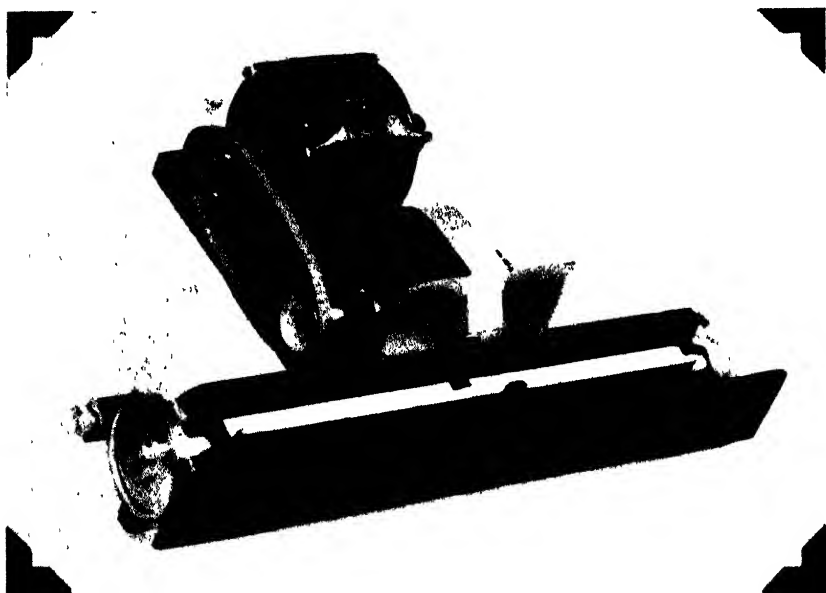


Fig. 1. A microtome knife sharpener employing a vertical glass wheel with a finely ground surface. An operator slides the knife back and forth across the narrow face of the wheel, at the same time holding the back of the knife firmly against the long L-shaped guide bar. See text for further details.

wheel is so slight as to be of no practical consequence. It will be seen that the objections cited by Funck to the horizontal disc do not apply to the vertical wheel provided the operator imparts a uniform lateral velocity to the knife. When this relatively simple skill is once acquired, the bevel can be abraded uniformly along its length, as demanded for sliding microtome knives. Stropping is unnecessary and it is best to dispense with it entirely. Polishing is normally done without any abrasive save the ground glass surface itself, with distilled water, dilute alcohol, or other liquid to prevent heating (cf. Garland, 1935). The operating speed may be high (1800 r. p. m.

for a 15 cm. wheel) provided the knife is moved rapidly enough across the wheel and the pressure against the knife is not too great.

The vertical wheel sharpener shown in Figure 1 was designed by the writer to accommodate the longer knives used on sliding microtomes and to facilitate the reconditioning of knives possessing a chisel shaped bevel. It differs from the Long model just described in size and in its general mechanical execution. The aim has been to attain the highest degree of accuracy and manipulative convenience compatible with simplicity and sturdiness of construction. The details of the mechanical design, many of which will be sufficiently clear from the accompanying photograph, will be concisely described.

A single brass casting weighing 23 kg. constitutes the entire framework for the apparatus including the base for the motor, the lower housing of the glass wheel, and the long trough (53 cm.) containing the guide bar assembly. In order that the abrasive suspension may flow back into its reservoir under the glass wheel, the bottom of the trough slopes down slightly from the ends to the center and the forward part of the apparatus stands on two legs, 5 cm. high. A stopcock on the bottom of the reservoir enables it to be emptied conveniently. The housing over the wheel is so fashioned as to direct an abundant supply of fluid to the point of contact between wheel and knife. To prevent possible splashing in the direction of the operator, an appropriate vertical projection has been designed on the front wall of the casting. The manner in which the steel guide bar unit has been assembled can be seen from the photograph. The bars of square (1.9 x 1.9 cm.) and rectangular (0.63 x 5.08 cm.) cross section are 45.7 cm. long, whereas the round cold rolled steel shafting is 2.54 cm. in diameter and 61 cm. long. The square bar has been recessed in the center to allow the closer approach required for small knives and to prevent splashing. Several small holes in the rectangular bar opposite the wheel permit a rapid drainage of the grinding fluid. To prevent rusting, all the steel parts have been "hard" chromium plated directly onto the steel in a hot solution, but how long such plating will resist abrasive action effectively is not yet known. The steel shaft is supported near the ends on V-slots in the brass casting, wherein it can be rigidly clamped by a partial turn of the thumb screw in the top thrust shown at the right end. Since the rotation of this shaft constitutes the sole operating adjustment of the sharpener, its accuracy of alignment is imperative. This accuracy can be attained in no better or simpler way than by the use of a round shaft in widely separated V-slots. A fine adjustment is furnished by means of a pinion and spur gear visible at the left end.

The positions of the guide bar are read on the circular scale and vernier mounted at the left end of the shaft. The circular scale consists of a flexible steel metric rule fastened on the periphery of a 10 cm. disc. Since bevel angles cannot be read directly in degrees where knives are of various dimensions, it is advisable to prepare a calibration chart for each size knife so that the bevel angles may be known.

In order to produce a chisel type knife edge or one having unsymmetrical bevels, the guide bar must be set alternately at either of two positions, corresponding to the two bevel angles desired, whenever the knife is reversed. To accomplish this, a cross bar, the range of motion of which is limited by two adjustable stops, has been attached to the extreme right end of the guide bar shaft (only the top edge of the cross bar is visible in the photograph). The stops consist of eccentric discs which can be clamped in any position, so that the operator need not adjust the settings on the vernier and scale before each reversal of the knife, but merely shifts between the two fixed positions.

The 1.59 cm. shaft for the glass wheel is mounted on high quality bronze bearings spaced 15 cm. apart. The desirability of mounting the glass wheel on its own shaft with its own bearings rather than on the armature of the motor has been emphasized by Prof. I. W. Bailey of the Bussey Institution, and Prof. Long has informed the writer that his forthcoming model will be so equipped. The advantages of such an arrangement, already amply demonstrated in a machine built at this institution several years ago by Mr. W. F. Court, Technical Assistant, are fourfold. It enables the bearings to be more easily protected from the abrasive suspension, favors accuracy in alignment, lowers replacement costs should bearings become worn, and leaves the motor free for other purposes.

The question naturally arises as to what further improvements are feasible on the vertical wheel sharpener. Would it be practicable to make it automatic? The extreme complexity involved in such a modification, and therefore expense, as well as the expectations of performance, seem to point to a negative answer. Even in a well executed and perfectly functioning automatic device, it is necessary to rely on actual inspection of the knife edge under the microscope. This means the attention of an operator. Furthermore, for critical and/or difficult sectioning it is most desirable to have the sharpener constantly available for frequent reconditioning of the knife by a few polishing strokes. This is more readily accomplished on a simple apparatus. Two conclusions follow: first, there is no ultimate substitute for "a little mechanical skill", and second, the sharpening process must be controlled by the one who uses the knife. Today, as in the day of von Mohl (1857), the critical worker "wird selten Gelegenheiten finden, seine Messer durch einen andern gut schleifen zu lassen."

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A MODIFIED WRIGHT'S METHOD FOR STAINING BLOOD SMEARS

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ABSTRACT.—After the blood smear is treated for the proper length of time with Wright's stain, neutral distilled water is used for diluting the stain. After the slide has been treated with neutral distilled water until the smear becomes pinkish it is then treated with pure absolute methyl alcohol which destains the plasma. Neutral distilled water is again kept on the mount until the corpuscles are well stained. Then the slide is dehydrated with absolute ethyl alcohol, cleared with clove oil and completed in the usual way.

Blood smears of different groups of vertebrates were uniformly brilliantly stained with the above technic.

Several lots of Wright's dry stain have been tested with the modified technic and no difficulties have been encountered in its application.

The well-known and commonly used Wright's stain for blood smears is a very satisfactory one. Sometimes there are difficulties, however, in securing uniformly successful results with the technic described by Wright (1902, 1910) and which have not heretofore, so far as I am aware, been overcome. Recently, Kingsley (1936) has referred to some of the unsuccessful results obtained with the Romanowsky (1890, 1891) types of blood technic, including Wright's, and has suggested a new stain for general hematological preparations.

The smears may be made in the usual way by placing a drop of blood on a clean slide and spreading it out by means of another slide, or a square cover glass. A much better method for making the smears has been used by the writer. A small sponge of fine texture is moistened with fresh blood and lightly drawn over the surface of a clean glass slide. With a little practice, it is possible to make a large number of good smears with a sponge which has been moistened only once with fresh blood.

Several lots of Wright's stain, including certified ones, have been tested by means of the modified technic, and all of them gave excellent results.

Blood smears of amphibians, reptiles, fish, birds, and mammals were made, and no difficulties were encountered with the technic.

Better results were obtained with the blood of certain amphibians with large corpuscles, such as *Necturus*, when the smears, before dry-

ing, were exposed for about a minute over the fumes of a 2% osmic acid solution. Better results were also obtained with such smears, if the slides were placed immediately upon drying into a Coplin jar containing pure absolute methyl alcohol. After such treatment for a few minutes, the slides were rinsed in neutral distilled water and stained at once.

The various steps in the technic are as follows:

1. Place 4 or 5 drops of Wright's stain on slide with blood smear for one to two minutes.

2. Dilute the stain with an equal number of drops of neutral distilled water. Allow to remain for about three minutes.

3. Drain slide and flood it with neutral distilled water. Replace the water when the smear appears slightly pink. Drain off the water.

4. Place slide for a few minutes into a Coplin jar containing pure absolute methyl alcohol. Instead of placing the slide into a Coplin jar with the methyl alcohol, a few drops of the methyl alcohol may be placed on the smear.

5. Remove the absolute methyl alcohol with neutral distilled water. Replace with more of the distilled water, and allow to remain on the stained smear until a pinkish color appears. Remove portions of smear not desired. Examine slide.

6. Dehydrate with absolute ethyl alcohol.

7. Clear with clove oil.

8. Follow clearing with balsam.

9. Place cover slip on stained smear.

The use of neutral distilled water is very important. Ordinarily freshly distilled water was sufficiently neutral for use. The pure absolute methyl alcohol removes at once the plasma stain, hence, a much better preparation may be obtained. The use of the absolute ethyl alcohol and clove oil insures a much more brilliantly stained smear.

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THE ANILIN BLUE COLLAGEN STAIN

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The anilin blue method for staining collagen fibrils and reticulum was published in 1900.¹ Later (1905)² the phosphomolybdic acid, used originally by itself as a separate step, was combined with the anilin blue solution and the oxalic acid in it was omitted. In this form the stain has been in general use ever since.

The method has three defects in consequence of which it has been variously modified. Most of the acid fuchsin on the market tend to fade in the sections owing to the deleterious action on them of the phosphomolybdic acid. Moreover, the stain with the anilin blue is not always so intense and sharp as could be desired. The third defect was that the method did not give so good results with paraffin as with celloidin sections. The three modifications most in use are Heidenhain's azocarmine, the Lee-Brown, and the Masson trichrome methods. All are somewhat complicated.

Recently I have again modified the method by replacing the phosphomolybdic acid by phosphotungstic acid and prolonging the time of staining in the anilin blue solution. The acid fuchsin solution may be made weaker or stronger according to the intensity of the red stain desired. The result is a clean-cut, intense blue stain of the connective tissue fibrils, reticulum and basement membranes.

The method now advised for general purposes is as follows:

Fixation in Zenker's fluid.

1. Stain paraffin or celloidin sections in a .25% aqueous solution of acid fuchsin for 30 minutes.

2. Drain and pass sections directly to the following solution:

Water.....	100 cc.
Anilin blue W. S.	0.5 g.
Orange G (85-90% dye content)...	2 g.
Phosphotungstic acid (Merck).....	1 g.

Stain for 1 to 24 hours, or longer if the deepest color obtainable is desired. An hour in the paraffin oven gives as good a result as overnight in the cold.

¹Mallory, F. B. A contribution to staining methods. *J. Exp. Med.*, 5, 15-20. 1900.

²Mallory, F. B. A contribution to the classification of tumors. *J. Med. Research*, 13, (N. S. 8) 113-36. 1905.

3. Transfer directly to 95% alcohol, two or three changes, until no more color is given off.

4. Absolute alcohol, xylol, neutral Canada balsam.

The method has been found to give excellent results with sections of formalin fixed pituitary glands. The acidophilic granules are stained bright red; the basophilic, blue.

TWO MICROTECHNICAL DEVICES

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1. A SIMPLIFIED MOUNTING CELL

Microscopists frequently mount small, thick objects such as pieces of cleared bone, whole mounts of embryos, and many specimens of general zoölogical interest. For mounting such material two devices are available: excavated slides and glass rings. Since both of these are rather expensive, technicians sometimes make a crude substitute for the manufactured glass ring by cementing on a slide small pieces of broken glass in the shape of a compartment which can be filled with balsam and over which a cover glass can be placed. The objections to this method are the time and labor required and the tendency of the balsam to escape between the pieces of glass. The common white celluloid curtain ring makes a very satisfactory substitute for the manufactured glass ring. Such a curtain ring can be cemented to a slide, filled with balsam, the object to be mounted inserted, and a round cover slip placed over it. Rough or warped rings can be smoothed by rubbing them on the side of a flat file, or enough cement can be used to compensate for the unevenness. Thick balsam makes an adequate cement. The only part of the mounting procedure which requires practice is judging the amount of balsam needed to fill the ring exactly so that when the cover glass is put on there will be neither overflow of balsam nor a deficiency resulting in air bubbles. Curtain rings may be procured at any dry goods or department store at a price not to exceed five cents a dozen. The size which measures $\frac{7}{8}$ inch in height, with inside and outside diameters of $\frac{5}{8}$ and $\frac{7}{8}$ of an inch, respectively, fits a $\frac{3}{4}$ -inch circular cover glass, and has proven very satisfactory.

2. A CONTAINER FOR HANDLING NITROCELLULOSE SECTIONS IN COPLIN JARS

A desire for economy of both time and material when paraffin and nitrocellulose sections are being stained simultaneously prompted evolving a technic in which ordinary Coplin jars are used for dyeing nitrocellulose sections. The essential instrument is a perforated test tube which fits easily into the jars. A soft glass test tube 15 cm. long and 2 cm. in diameter is perforated with a hot 20-gauge platinum

¹Contribution No. 230.

wire, altho hole size may be altered with variation in section size. If the holes are too large there is danger of the sections escaping. It is sufficient to have 5-12 holes near the bottom. One hole should be pierced at the very bottom of the tube to insure adequate drainage. Cracking of the tube may be avoided by heating and blowing the end enough to thin the glass prior to perforation. The sections to be stained are put into the perforated tube, and the latter is transferred from bath to bath. By wiping the tube and blowing the excess dye from it between jars, the contamination of the succeeding solution does not exceed that effected by a slide. As many as fifty sections have been stained at once with absolute uniformity of stain, even in short-time baths of high dye concentration. Staining homogeneity can be assured by stirring the solution in the tube with a suitable rod, or, more simply, by the application of gentle oral suction to the open end of the tube. Sections are most easily extracted from the tube by a stiff, flat paint brush $\frac{3}{8}$ -inch in width.

A TECHNIC FOR CLEARING LARGE INSECTS

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The following schedule has been found to clear large insects successfully. Highly pigmented animals and specimens with chitinous coverings are rendered translucent by this method. Hence, finer structures of the external body wall may be studied to advantage.



Fig. 1. Photograph showing two grasshoppers, the one on the left is untreated; the one on the right has been cleared by the technic described. Magnification approximately $2\times$.

Some dyes, e. g., picro carmine and borax carmine are not washed out by the various reagents, with the result that the organs which take the stain may be rendered more conspicuous. The two photographs demonstrate the effect produced by this technic.

The method is as follows:

1. Absolute alcohol, 5 to 15 days.
2. Alcohols, 95%, 85%, 70%, 50%, about 15 minutes each.
3. Alcohol, 35%, 30 minutes.
4. H_2O and H_2O_2 (50-50) plus trace of NH_4OH , 12 to 24 hours.
5. Alcohols, 35%, 50%, 85%, 95%, about 15 minutes each.
6. Absolute alcohol, 2 or 3 changes, at least 3 days.
7. Toluol, 10 days to 3 weeks.

The specimen may then be mounted in some suitable medium such as



Fig. 2. Photograph of a fruit fly, *Drosophila*, which has been cleared by the technic described. Magnification approximately 40 \times .

dammar. This is best accomplished by passing gradually from thin to thick dammar so that there is a thoro penetration of the medium. If the dammar is dissolved in xylol, the specimen should first be passed thru the latter before proceeding to the dammar. Benzole or xylol may be used in place of toluol, but either makes the material more brittle than does toluol.

If rings of gum rubber are cut and dropped in xylol for a short time, they become hard and afford a good support for cover glasses in the mounting of larger insects.

POSSIBLE USES OF DIOXAN IN BOTANICAL MICROTECHNIC

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ABSTRACT.—Dioxan has been well established as an advantageous dehydrating agent for plant tissues. It dehydrates equally well after fixatives containing formalin, acetic acid, chromic acid, chromates, mercuric chloride, osmic acid, and alcohol. Better infiltration of paraffin after dehydration may be obtained by passing the material thru (1) a cold bath composed of 30 cc. of dioxan, 5 cc. of xylol and 20 cc. of melted soft paraffin and, (2) a warm bath of 50 cc. of dioxan, 50 cc. of paraffin, and 10 cc. of xylol. Transfer from (2) to soft paraffin. A dioxan fixative consisting of dioxan 50 cc., formalin 6 cc., acetic acid 5 cc., water 50 cc. was devised for delicate subjects. The fixed material is transferred directly into dioxan and mounted in dioxan-diaphane or dioxan-balsam. Very delicate objects require dioxan dilution of the balsam and slow concentration of the mounting medium by evaporation.

Entire plant parts or epidermal peelings are fixed in any desired fixative, washed if necessary, transferred to dioxan and mounted in diluted dioxan-balsam or diaphane. Dioxan may be used to mount hyalin objects whose refractive indexes approach those of balsam in media of higher index than balsam. It may be used in place of alcohol in finishing paraffin sections, and since it exhibits different stain solubilities than alcohol it offers an important new tool in obtaining and maintaining stain balances.

Since the publications by Graupner and Weissberger (1931 and 1933) recommending the use of dioxan (diethylene-oxide)² as a substitute for alcohol and xylol in microtechnic processes a few American workers have observed the advantages of this chemical. Johansen (1935) discussed the desirability of dioxan and tertiary butyl alcohol as dehydrants for plant tissues but did not elaborate upon the details of his technic. Recently Baird (1936) discussed the application to animal tissues. From nearly two years' experience with dioxan in botanical microtechnic in this laboratory we feel that dioxan both simplifies and improves paraffin sectioning and that it offers new fields or possibilities in microtechnic. This paper presents schedules

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²We obtained diethylene oxide from the Carbide and Chemical Corporation, under the name dioxan.

adaptable to plant tissue embedding, and points out some of the special applications of dioxan.

SCHEDULES FOR THE PARAFFIN METHOD

Fixation: We have used dioxan with a number of standard botanical fixatives comprising mixtures of formalin, acetic acid, chromic acid, and alcohol, and with a few cytological fixatives including Flemming's, Helly's, and certain mitochondrial technics. The choice of fixative does not limit the use of dioxan, but chromic acid mixtures do seem especially suitable for dioxan dehydration.

Washing: Except where tissue has been darkened (strong chromic or Flemming's) elaborate washing is unnecessary, rinsing off the excess fixative being sufficient. After washing or rinsing, transfer to undiluted dioxan.

Dioxan containers: When considerable embedding is to be done, the original recommendations of Graupner and Weissberger (1931) are advisable. The process consists of using the dioxan in a wide-mouthed glass-stoppered cylinder; the lower part of the cylinder is filled with anhydrous calcium chloride held in place with a fine-meshed wire screen. The principle involved is that the dioxan removes the water and fixative from the tissue and the calcium chloride removes both from the dioxan. One cylinder of dioxan and calcium-chloride will suffice for over one hundred embeddings. Tea-balls are convenient for holding the tissue in the liquid. When only a relatively small amount of embedding is to be done the somewhat "fussy" calcium chloride cylinders are unnecessary. In this case the dioxan is used as one would alcohol: simply place the fixed tissue (after a short washing) into a small vial, cover well with dioxan and change once or twice to insure complete dehydration. The cylinder method is advantageous and economical for handling large amounts of tissue, or for demonstrating the technic to students; the vial method for the individual worker who makes paraffin sections only occasionally. Dioxan containers should not be used or kept on any painted surface since the chemical is an exceedingly active paint remover.

Dehydration: After rinsing off the fixative pass the tissue directly into undiluted dioxan. In our tests grading dioxan as one would alcohol proved to be of no advantage. The time in dioxan will naturally vary with the size of the tissue. Three hours is a satisfactory time for small pieces not more than one millimeter thick, six hours for larger pieces. Two hours is the shortest time we have tried, but that was with very small pieces of tissue. Since tissue does not materially harden in dioxan one may leave the tissue in dioxan for several days without affecting the efficiency of the pro-

cess—a convenience to one who has to pick the time he can conveniently embed.

Embedding baths: While dioxan is only a weak solvent of paraffin, it is readily miscible with paraffin. One may transfer from dioxan directly to soft paraffin, but trial embeddings of thick leaf tissue have shown that it is advantageous to add a small amount of xylol at the beginning of the embedding series. Two special baths of paraffin mixtures have been standardized in this laboratory:

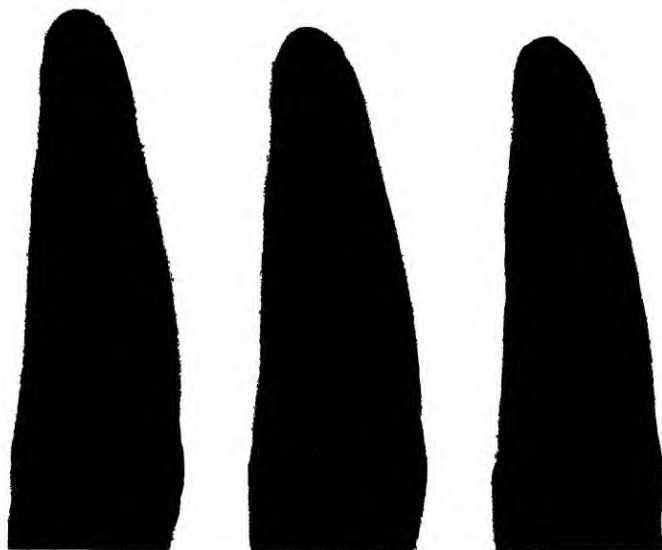


Fig. 1. Sections of narcissus leaf cut parallel to surface. These large sections cut with rotary microtome were possible because the leaf blade remained flat during the dioxan-paraffin embedding process. 72 mm. microtessar projection at 4 \times .

(1) To 50 cc. of dioxan and 5 cc. xylol add 20 cc. of melted, soft paraffin. This produces a flocculent-like mixture. Allow this mixture to stand until it assumes room temperature and forms a floating "cake". Then pour the liquid from under the paraffin into a suitable container. This becomes paraffin bath No. 1 and is used at room temperature. On cold days it may become turbid and should then be warmed before using.

(2) To 50 cc. of dioxan add 50 cc. of melted paraffin and 10 cc. of xylol. This bath is used at 50° C., or whatever temperature one chooses for soft paraffin.

Embedding procedure: Tissue is transferred quickly from dioxan into small vials in which paraffin mixture No. 1 is immediately poured.

The vials are placed in a vacuum chamber for 10 to 30 minutes. Mechanical removal of air greatly improves penetration into plant tissues where large air spaces are present even if the tissue was vacuumed during fixation. The total time in bath No. 1 need not be more than 30 minutes. The liquid (No. 1) is then poured off and bath No. 2 at 50° C. is quickly poured in, and the vials placed in the oven. The time in bath No. 2 need not be more than an hour.

From No. 2 transfer directly to pure soft paraffin, to hard paraffin if necessary, and embed as usual.

Some of the advantages of dioxan for the paraffin method are:



Fig. 2. Cross section (paraffin) of edge of leaf of *Vallota*, a thick-leaved member of the *Amaryllis* family. The solvent action of dioxan does not interfere with the preserving and recording of cuticular coverings. These thick-walled epidermal structures become brittle in alcohol-xylol embedding but are easily cut by the dioxan process. Stained in safranin. Photographed with B filter. Zeiss 8 mm. N. A. 0.65. (400 X)

(1) Washing after fixation is largely eliminated. Dehydration and washing take place simultaneously. Graupner and Weissberger (1931) pointed out that even those fixatives containing picric and trichloroacetic acid can be "washed out" with dioxan-calcium-chloride.

(2) The tedious alcohol-xylol gradation series is completely eliminated. There is only one step between fixation and infiltration. Infiltration is usually accomplished without any plasmolysis and with far less plasmolysis than the average carefully graded alcohol-xylol series permits.

(3) There is little hardening of tissues. Johansen (1935) attributes this to dehydration without desiccation. See Fig. 2.

(4) Tissues show much less tendency to curl than in alcohol and xylol. See Fig. 1.

(5) Speed and convenience: After fixation the tissue can be left

in dioxan for three hours or until one is ready to embed. Plant materials left over 12 hours become clear but do not harden.

(6) Material passed thru dioxan into paraffin can be softened in water for sectioning. A technician working under our direction cut thick canes of *Ribes* by this process. Johansen has noted this point and utilized it for cutting wood sections.

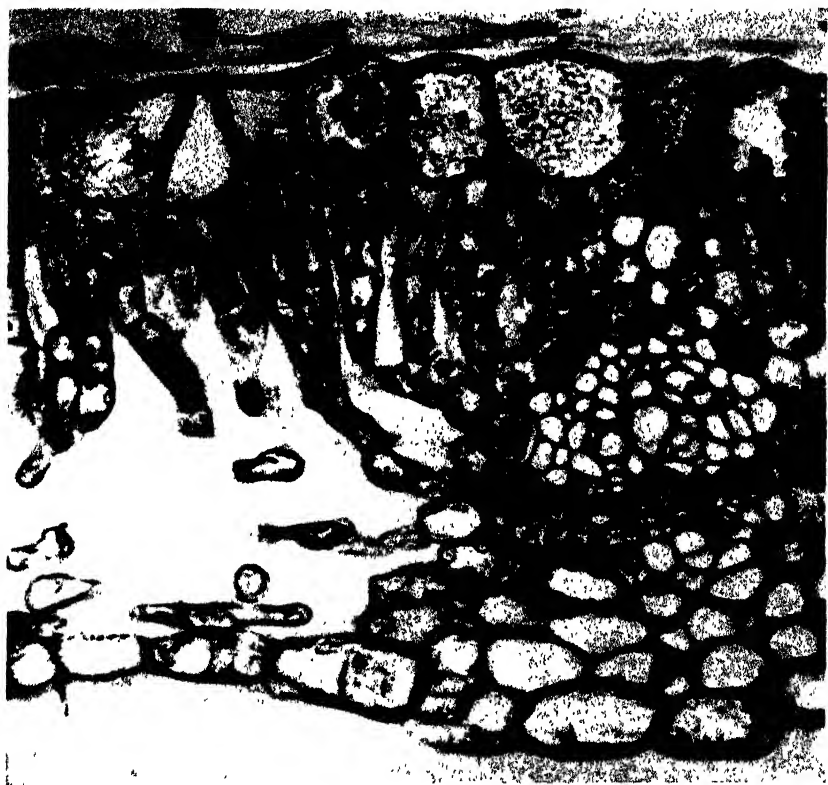


Fig. 3. Cross section (paraffin) rose leaf attacked by powdery mildew (*Sphaerotheca*). Two lobed-haustoria are visible in the epidermis. The epidermal cells and those of the pericycle contain gums and red pigments. This slide is faintly stained in safranin to facilitate rendering the subject in monochrome. Photograph shows how the extremely varied cell contents are retained during the dioxan process. B filter. Zeiss 4 mm. 0.95 N. A. (600X)

(7) It is an inexpensive method. One liter of dioxan is sufficient for at least one hundred embeddings.

Some of the disadvantages of dioxan for the paraffin method are:

(1) Very soft tissues remain too soft for satisfactory embedding. For example, partly ripened grapes remained too soft and failed to embed properly.

(2) Penetration by paraffin is not always satisfactory. This can usually be overcome by increasing the amount of xylol in the first paraffin bath. Tissues that are difficult to penetrate by the dioxan method are likewise difficult subjects for the standard alcohol-xylol method.

The above statements are based on the experience of making about 800 research slides representing about 160 separate embeddings, and on trials by students in technic courses.



Fig. 4. Young "net" of the alga *Hydrodictyon* mounted in Diaphane after fixing in dioxan fixative (page 111) and dehydrating in dioxan. Unstained. Photographed with Zeiss 3 mm. N. A. 1.00 lens. H filter, $\frac{1}{8}$ light cone. (800 \times)

SPECIAL USES AND METHODS

1. MOUNTING DELICATE MICROSCOPIC SUBJECTS IN BALSAM

Dioxan is an astoundingly efficient dehydrating agent for mounting delicate objects in balsam or balsam-like media. Johansen was the first to mention this use. We had independently developed this technic and concluded that such usage of dioxan is its most important and unique application. It was practically impossible, however, to fix some protozoa, the algae, *Hydrodictyon*, and spores of fungi without slight plasmolysis with any fixative we tried. Dioxan treat-

ment did not increase the plasmolysis. This suggested the possibility of including dioxan in the fixative. The following formula was devised:

Dioxan	50 cc.
Formalin	6 cc.
Acetic acid	5 cc.
Water	50 cc.

This fixative has permitted balsam or diaphane mounts of *Vorticella*, *Hydrodyction*, *Mucor*, nematodes (several kinds) freehand



Fig. 5. Spores of *Cy lindrocarpon* (a *Fusarium*-like fungus) from agar culture. Dioxan fixative, dioxan processed. Stained in Ehrlich's hematoxylin to demonstrate nuclear content. Late stages of mitosis are shown in the central spore. Zeiss 5 mm. 1.40 N. A. (1800 \times)

sections of mushrooms dropped directly into the fixative, mites, and many other subjects which are usually difficult to mount even in glycerin. In many of these preparations magnifications of 1,000 times fail to show a trace of plasmolysis. See Fig. 4 of *Hydrodyction* and Fig. 5 of fungus spores.

Fix ten minutes to one hour in dioxan fixative. Transfer to undiluted dioxan, or if advisable to a stain³ dissolved in 50% dioxan,

³Staining methods for this particular purpose will require special investigation. Methyl blue in 50% dioxan is one of the most promising yet tried. Most stains are only slightly soluble in dioxan thus permitting dehydration with little stain removal. This factor makes it difficult to stain in dioxan without running the material back to water.

and then to pure dioxan. Place a few drops of very dilute Canada balsam dissolved in dioxan, or in diaphane diluted with dioxan, in a watch glass. For delicate subjects the mounting fluid must be water-like in dilution; use about ten parts of dioxan to one of balsam. Place specimens directly into this; allow the preparation to evaporate at room temperature until it assumes a syrup-like consistency. Transfer to a drop of dioxan-balsam or diaphane of about the same consistency and cover, adding thick balsam in dioxan to the edge of cover, as needed.

The presence of xylol in the balsam is objectionable for two reasons:

(a) If no xylol is present, traces of water which may have remained in the tissue, escape into the dioxan balsam. Dehydration continues after mounting!

(b) If the slide proves unsatisfactory and no xylol is present, the balsam is easily removable by washing in water!

Very small objects can be run thru on a slide or cover. We frequently make mounts of fungus cultures in the fixative instead of in water. Then if it seems desirable to preserve the mount, dioxan is drawn under the cover, then dioxan-balsam, and the mount is preserved.

2. MOUNTING DELICATE OBJECTS IN MEDIA OF HIGH REFRACTIVE INDEX

It is well known that mounting media frequently obscure details because the refractive index of the medium is too close to that of the object. This is especially true of glycerin preparations where the index ranges between 1.40 and 1.46. Sugar solutions such as "Karo" with an index around 1.48 are easy to use but have the same objection. Canada balsam, $1.50 \pm .02$ is slightly better but requires staining of objects for optimum vision even with critical illumination. Water mounts, with the index 1.33 well below that of the object, offer for critical illumination methods objective fields in which a hyalin object shines in a light ground—but such mounts are not permanent. By dioxan infiltration it is possible to mount objects in synthetic gums or resins such as "Hyrax" with a refractive index greater than 1.80. In such a medium objects which are hyalin in water appear as dark bodies on a light ground. At the present time we are having made special lots of "Hyrax" material for perfecting this method of mounting. It is probable that such a method will eliminate the necessity for staining where the form, shape, and size of the object are the chief considerations. These conditions obtain

in the average mycological preparation. The effect of the medium on resolving the outline of the object is shown photographically in Fig. 7.

3. MOUNTING ENTIRE PLANT PARTS AND EPIDERMAL PEELINGS

The process for mass tissues is essentially the same as that outlined in No. 1 but for some reason tissues are far less prone to plasmolyze than filamentous fungi and algae. They may, therefore, be handled with fewer precautions and with a larger variety of fixatives. The following procedure is efficient and rapid for soft plant tissues such as portions of leaves or slices of herbaceous stems and roots.

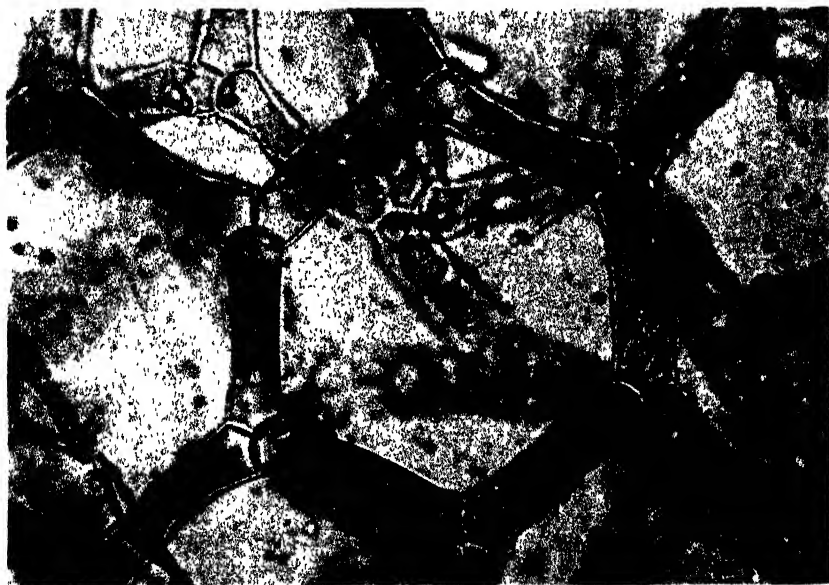


Fig. 6. The whole mount method applied to x-body differentiation. Subject, *Hippeastrum equestre*, virus diseased (type material plants supplied by Dr. Kunkel) leaves sliced parallel to epidermis, (i. e. "chunks," not machine sections) fixed in acetoformalin, faintly stained in Ehrlich's hematoxylin (after mordanting as for iron hematoxylin) counterstained in gentian violet dissolved in 50% dioxan, transferred to pure dioxan, cleared (in dioxan) for 18 hours, then mounted in Diaphane diluted with dioxan. Photographed: Zeiss 3 mm., 1.40 N. A. on process pan. The picture represents an optical section of the nucleus and its plasmosome. Chromatin granules of the resting nucleus are clearly shown. The tail-like material subtended by the nucleus is a partly organized x-body. (1200X)

Fix in any desirable fixative. Five per cent acetic acid, 6% formalin in 50% alcohol, mixed when used to avoid formation of esters, is usable for almost any tissues.

Transfer directly to pure dioxan or to a stain, and then to dioxan. The minimum time in dioxan is 30 minutes, but if the tissue is left for several hours it will become very clear and permit better vision of the inner cells.

Mount in dilute dioxan-balsam or diaphane. It is not necessary to use very thin balsam and evaporate it down in this case.

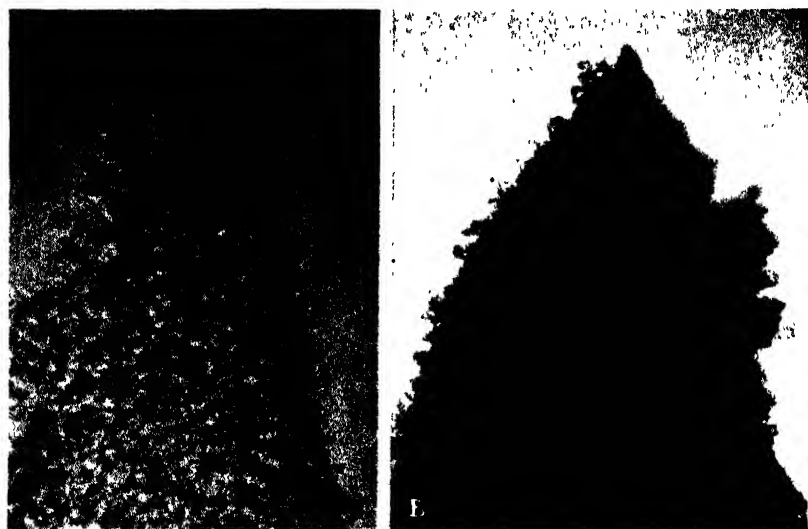


Fig. 7. Photographs of small bits of mushroom (*Leptonia*) gill mashed flat, fixed in dioxan-fixative and dioxan dehydrated. (A) mounted in Canada balsam (Diaphane) (B) mounted in Hyrax. These are photographed with a Zeiss 8 mm. 0.65 N. A. lens using exactly the same light cone ($\frac{2}{3}$) in each case and commercial Panchromatic film. The difference in appearance and in rendering of basidia at the edges is due entirely to the refractive index of the medium. Neither are stained and the tissue is very hyalin. This high refractive index medium (above 1.80) were designed for diatom shells and similar non-plasmolizable material, but by dioxan processing they may be used to show the form of delicate hyalin objects. Prints on "contrast" vitava paper carefully balanced to show relative contrast of negatives. (400 \times)

This method is especially useful for leaves. Cytological details may be readily followed and gross anatomy is visually charted. If stained in light green, they appear as living entities, but are more visible within than are living tissues because of the clearing. Permanent mounts of epidermal peelings and portions of leaves have proven efficient for studying x-body inclusions within tissues from virus diseased plants. (Fig. 6).

4. MITOCHONDRIAL AND PLASTID TECHNIC

Dioxan is efficient in connection with mitochondrial technics. Altho a powerful solvent of fats and lipoids, it does not appear to remove them from cells where they have been subjected to saponification with chromates. The usual procedures of fixation are followed, the material is washed and then dehydrated in dioxan. Cytological details within growing points and leaves are well preserved.

Epidermal peels fixed in mitochondrial fixatives, washed, stained, dehydrated in dioxan and mounted in balsam-dioxan give excellent preparations of plastids without recourse to tedious alcohol dehydration or to paraffin sectioning. Other than special fixation the technic is essentially that presented in No. 3, above.

5. RUNNING SECTIONS DOWN FROM XYLOL AND STAINING

Dioxan can be used to replace most of the alcohol series in running paraffin sections down from xylol. The important difference in stain solubility existing between alcohol and dioxan will permit the development of new stain technics and simplification of existing technics where the extreme solubility of some stains in alcohol causes difficulties in finishing slides. These stain solubility differences offer an important field for investigation.⁴

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⁴In the synthetic organic chemical catalog of Carbide and Carbon Chemicals Corporation it is stated "The oil soluble dyes are soluble in dioxan while the water-soluble and spirit-soluble varieties are practically insoluble."

A NEW STAINING TECHNIC FOR PERITHECIA OF THE ERYSIHACEAE

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Any process involved in the staining of perithecia should be simple and yet satisfactory from the standpoint of detail.

It was our idea that a good bacteriological stain designed for the penetration of hardened cell walls of certain bacteria could likewise be used to penetrate peridial walls. While the bacteriologist uses a short-period heating process for penetration of stain, we used a long-period process (48 or more hours). Our theories regarding stain penetration seem to be justified as the results desired were all present. In the following schedule perithecial wall, asci, ascospores, nuclei, and even appendages were well stained. Care in handling will eliminate danger of breaking off appendages.

Dry perithecia carefully scraped from the leaf of the host should be placed directly in Ziehl's carbol fuchsin for a period of 48 hours or more, in an oven kept at 50° C. Some of the perithecia carefully removed from the staining vial should be placed on a clean slide and covered, and the perithecia opened by tapping, smartly, on the cover slip. Slides bearing opened perithecia should then be heated for 15 minutes in an oven kept at 95° C., with stain constantly being added to replace that being steamed off. A drop or two every three or four minutes placed at the edge of the cover slip will suffice. Excess stain around the cover slip should be carefully removed before the destaining process begins. For destaining acid alcohol (2% solution of concentrated HCl in 95% alcohol) must be drawn rapidly under the cover glass by means of a blotter or filter paper. We have found that six to ten drops of acid alcohol will suffice for destaining, after which the stained organisms are washed by allowing 95% alcohol to be drawn under the cover slip. The process is complete after the perithecia are washed in a series of 25%, 50%, and 75% xylene in 95% alcohol, followed by pure xylene. Since the perithecia adhere to the slide, the cover slip may be removed while the balsam or hyrax is being added.

DETAILS OF TECHNIC

1. Dry perithecia should be stained in Ziehl's carbol fuchsin (fuchsin 1 g., carbolic acid crystal, 5 g., 95% alcohol 10 cc., H₂O 100 cc.) for a minimum of 48 hours in an oven at 50° C.

¹Mr. Glenn L. Hays aided in preparation of stain.

2. Place perithecia on a slide and open with cover slip.
3. Transfer to oven for 15 minutes at 95° C.; evaporating stain to be replaced by more stain.
4. Remove with acid alcohol (2% solution of concentrated HCl in 95% alcohol) all excess stain left on the slide.
5. Destain with acid alcohol; it is well to watch this process under the microscope.
6. Wash thoroly with 95% alcohol.
7. Wash with 25%, 50%, and 75% xylene in 95% alcohol, followed by pure xylene.
8. Mount in balsam or hyrax.

NOTES ON TECHNIC

A DIOXAN TECHNIC.—In recent years the classical technic in cytology has undergone some important changes which have tended not only to hasten processes but to improve results. These advances have been made possible thru the use of two substances, i. e., butyl alcohol and dioxan. The latter substance has some valuable properties which give promise of making it one of the most important agents in microscopical technic. Recently I have extended the use of dioxan and have thereby been able to simplify and shorten the process of making sections by the paraffin method. In this new technic the tissue never leaves dioxan solutions, unless for staining, from fixation until embedding and after sectioning is mounted in a dioxan medium. Changes are reduced to a minimum and treatment with harsh media is avoided, thus lessening hardening and shrinkage of delicate materials. Briefly the steps are as follows:

1. Fix in a mixture of one part of dioxan with 2 parts of a picromol-acetic solution (e. g., Bouin, or one of the writer's modifications thereof.)
2. Wash in dioxan.
3. Dehydrate and clear in fresh dioxan.
4. Infiltrate with warm mixtures of paraffin and dioxan, with increasing proportions of paraffin.
5. Infiltrate in paraffin.
6. Cut, spread and dry sections.
7. Decerate in xylene or dioxan.
8. Stain.
9. Wash.
10. Dehydrate with dioxan.
11. Mount in sandarac dissolved in dioxan, the concentration varying with the type of tissue, and to be determined by experience.

Dioxan dissolves picric acid readily, but does not dissolve many of the anilin dyes tried. It is a partial solvent for water, alcohol, xylol, paraffin, sandarac and dammar. The milky solution of dammar can be cleared by the addition of a quantity of xylol. By the addition of camsal (a fluid produced from a mixture of camphor and salol) to the sandarac solution in dioxan, a mounting medium similar to euparal is produced. Material can be left in dioxan for several weeks without injury. Infiltration in paraffin is slower after dioxan than after xylol, but tissues do not suffer from the heated paraffin.

Dioxan is more expensive than alcohol, but by making use of a series of baths very little need be lost and in this way it costs no more to use than ordinary reagents. By using fixing fluids with dioxan, acetic acid and chloroform, or with dioxan, acetic acid and formalin, a quicker result can be obtained than with picric acid. Fixed materials stored in 70% alcohol can, of course, be carried up into dioxan, sectioned and mounted according to the suggested method.—C. E. McCLUNG, Univ. of Pennsylvania, Philadelphia.

THE REFLECTING PRISM IN MICROSCOPY.—H. C. Waterman, in his notes in *Stain Technology* (January, 1934), deplors the prohibitive price of the reflecting prism recommended by Belling in place of the microscope mirror. Thrifty readers may, perhaps, benefit by my own experience. Recently, I noticed a heterogeneous collection of prisms in an optician's window in England which, I was informed, had been salvaged from derelict instruments. I had no difficulty in securing a perfect specimen of ample dimensions for the equivalent of two dollars. It seems probable that many opticians carry similar collections.—D. B. O. SAVILE, Macdonald College, P. Q., Canada.

A SIMPLIFICATION OF THE PARAFFIN EMBEDDING PROCESS.—The use of the electric refrigerator and rubber ice-cube trays in embedding with paraffin has been a means of saving time and obtaining more translucent blocks. The rubber type of tray is more practical than the metal type since it is pliable enough to allow the removal of the blocks after hardening. Altho they form only one size of block, this is large enough to contain good sized specimens, and yet does not form a permanent waste of paraffin around the smaller ones, since paraffin shreds may be remelted if the clearing agent has been removed before embedding.

The use of the electric refrigerator allows the regulating of the temperature needed to cool the blocks quickly and evenly. Small paper labels floated on the hot paraffin in the trays form adequate means of identification for each block.—HARRIS P. MOSHER and Miss E. C. ADAMS, Mosher Laboratory, Massachusetts Eye and Ear Infirmary, Boston, Mass.

A WORD OF CAUTION CONCERNING DIOXAN.—Since the publication of the article "Comparative Study of Dehydration", (*Stain Technology*, 11, 13-22, 1936), the attention of the author has been directed to the possible toxicity of dioxan. The *Industrial Chemist*, (No. 122, 11, 1935) states that the effects of inhaling dioxan are cumulative and result in pathological damage to the liver and kid-

neys. Prolonged exposure in concentrations 1 in 1000 produce severe toxic effects.

Caution in the use of this reagent is, therefore, necessary. It is not likely, however, that such industrial conditions will be duplicated in the technical laboratory where only small quantities of the reagent are used and mostly in closed bottles.—T. T. BAIRD, Columbus, Ohio.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the April number of this journal.

STAINS CERTIFIED MARCH 1, 1936 TO MAY 31, 1936*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Basic fuchsin	DF 4	91%	For use in bacteriological media	Mar. 6, 1936
Congo red	LQ 5	75%	As histological counter-stain	Mar. 12, 1936
Basic fuchsin	NF 28	84%	As bacterial stain and for use in bacteriological media	Mar. 13, 1936
Methyl violet 2B	NMv 5	85%	As bacteriological, histological and cytological stain	Apr. 1, 1936
Basic fuchsin	NF 3	91%	As bacterial stain and for use in bacteriological media	Apr. 1, 1936
Giemsa stain	GGe 3	—	As blood stain	Apr. 17, 1936
Janus green B	CJ 3	50%	As vital blood stain	Apr. 17, 1936
Giemsa stain	NGe 2	—	As blood stain	Apr. 21, 1936
Crystal violet	LC 10	92%	As bacteriological, histological and cytological stain	May 5, 1936
Eosin B	NEb 7	90%	As histological counter-stain	May 6, 1936
Methylene blue	NA 11	86%	As histological and bacteriological stain; and as constituent of blood stains	May 22, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

MICROSCOPE AND OTHER APPARATUS

HERTIG, M. Hollow-ground slides for whole mounts made with the dental engine. *Science*, 83, 110. 1936.

The dental engine (motor, flexible shaft and grinding tools) proves to be a useful implement for making slides with all manner of depressions to fit any specimen. This permits resting the cover glass upon the slide, allows saving of the mounting medium and facilitates orientation of the specimen. Of the tools, the abrasive wheels mounted on a mandrel are perhaps the most generally useful. Grinding may be done rapidly in a drop of water. A depression for a mature flea larva will take 10-15 sec., for a mosquito larva 30 sec., for a bedbug 1 min.—*J. A. de Tomasi*.

SCHUMACHER, J. Eine einfache Methode zur Angleichung der Bild- und Zeichenflächenhelligkeit bei Zeichenapparaten und -okularen. *Zts. wiss. Mikr.*, 51, 392-3. 1935.

While sketching at the microscope, the usual way of equalizing the intensity of illumination of the microscopic field and the sheet of drawing paper consists in the interpolation of gray filters. This is often unsatisfactory, owing to the crudeness and other shortcomings of the filters. It is suggested that one extra lamp be supplied for the drawing board, with a variable resistance placed in the line at the left of the microscope. During the process of drawing, with the camera lucida in position, the left hand can operate the resistance knob so that the lighting can be equalized at will. Increasing or decreasing the illumination of the sketch also allows immediate comparison of the object and the drawing.—*J. A. de Tomasi*.

PHOTOMICROGRAPHY

BERTHELSEN, H. Eine objektive Methode zur Bestimmung der genauen Expositionszeit bei der Mikrophotographie. *Zts. wiss. Mikr.*, 51, 383-7. 1935.

A method is described for the determination of the correct exposure time in photomicrography. It is based on the use of a photoelectric exposuremeter, the "Metraphot" built by Metrawatt Nürnberg A. G. The apparatus cannot give a direct reading at the microscope, but the exposure may be calculated by correlation. It is claimed the method affords a saving of time and plates.—*J. A. de Tomasi*.

JORDAN, H. A new apparatus for the daylight projection of microscopic and lantern slides. *Science*, 83, 167-8. 1936.

This simple screen or reflecting box permits projection of lantern slides, photographs, and plates in plain daylight. It consists of a box with sides sloping outwards, its farther face covered with glazed or aluminum-coated paper. The rest of the interior is painted black. It has proved of value in demonstrating to small groups of students.—*J. A. de Tomasi*.

MICROTECHNIC IN GENERAL

CASTELLENGO, L. Bobine pour rubans de coupes en série. *Bull. d'Histol. Appl.*, 12, 323-8. 1935.

This appliance consists essentially of a spool, controlled by a drum and axle, which is held with screws and brackets parallel to the microtome blade. The ribbon is wound by hand around the spool as soon as made. There are several advantages claimed, chiefly the production of continuous ribbons which should eliminate variations in thickness and loss of any sections. A series of 248 sections, 7 μ thick, 8 mm. wide, was cut in 48° C. paraffin. In spite of the 2 m. length of the ribbon and of 23° C. room temperature, no inconvenience was experienced.—*J. A. de Tomasi.*

COX, A. J., JR. A simple basket carrier for use in tissue dehydration. *J. Lab. & Clin. Med.*, 20, 1298-99. 1935.

Cut out a piece of copper window screen 3½ x 2½ in., and a smaller piece about 1½ in. square. Two wires are removed from each end of the large piece. Roll this into a cylindrical shape, interlace and tie the protruding ends. Remove two wires from one end of the cylinder thus formed and attach the smaller piece of screen to make the bottom. The resulting basket measures 1 x 2¼ in., is quite cheap, and can be used for fixation as well as embedding.—*J. A. de Tomasi.*

ETKIN, W. A simplified procedure for the volumetric measurement of serially sectioned structures. *Science*, 83, 214. 1936.

The volume of small or diffuse glands can be successfully computed by drawing (with the aid of a projector or a camera lucida) the outline of sections in sequence on a continuous sheet of wrapping paper. A draftsman's planimeter is set upon an ample sheet of clear flat material such as celluloid. A pinhole at the center marks the starting point. The sheet with outlines is slipped under the celluloid and a point on each perimeter is brought to coincide with the starting point. Without disturbing the planimeter the pointer is traced around and the final reading gives the total sum of the areas. From this and the thickness of the section, the volume is calculated. The volume of a gland spread over more than 100 sections can be estimated in less than 2 hr.—*J. A. de Tomasi.*

GOOSMANN, C. Dark-field illumination in the diagnosis of tuberculosis and malaria. *J. Lab. & Clin. Med.*, 21, 421-4. 1936.

Dark-field illumination is not only useful for the study of live organisms, but can also find application in the examination of stained slides. Thus tubercle bacilli appear much brighter and bigger, while the blue stained non-acid-fast bodies are almost invisible. The same is true of the malaria plasmodium in Giemsa stained blood smears. It should be easy to switch from dark-field to transmitted light illumination: all that is needed for this is a "center stop" to be inserted below the Abbe condensor. The field is searched with a dry condensor; a drop of water between condensor and slide will then give a better dark-field. As the size of the center stop depends on the aperture of the objective and on the ocular, it is well to keep several sizes on hand. The "Mikropolychromar" of recent introduction is based on the same principle: the dark center disc gives a blue background, while the lighter rim colors the objects bright red.—*J. A. de Tomasi.*

GRAFF, J. H., ULM, R. W. K., and HOWELLS, T. A. The factors involved in the accuracy of fiber analysis. *Paper Trade J.*, 101, No. 2. 1935.

A standard method is given for preparation of slides for fiber analysis of paper and making the fiber count. Results are accurate within less than 2% when two fields are counted, and should be reported in exact figures, not to the nearest 5%.

Cover 0.5 g. of the paper with 1% NaOH in a 30 cc. beaker and bring to boil on a hot plate. Decant and wash twice with water. Cover with 0.2 N HCl, let stand several minutes, decant and wash several times with water. Drain, roll into pellets and disintegrate by shaking in a flask with 250 cc. water. Measure 125 cc. of the suspension into a 250 cc. graduated cylinder, dilute with water to 250 cc. and mix. Pour off 125 cc., dilute again to 250 cc. and mix. This makes a 0.05% suspension.

Slides kept in 0.5% HCl in 50% alcohol are dried and polished. With a wax pencil draw a line across the slide 1 in. from each end to keep the suspension within end areas. Place on a level hot plate regulated to 50° to 60° C. Pipet 0.5 cc. of suspension onto each end of the slide. Evaporate partly, and tap to distribute uniformly. Then dry completely. Remove and add 2 to 3 drops of stain (particular stain not given); cover; let stand 1 min.; and drain on blotting paper.

Count intercepts of fibers with cross hair on 5 traverses across the slide 5 mm. apart, beginning 3 mm. from either end. Fine fragments are neglected, larger ones are counted as $\frac{1}{2}$ or $\frac{1}{3}$. Where 2 fibers are being counted, count both while going in one direction, but only one in the reverse direction. Determine second fiber by difference.—*E. Venable*.

KERNOHAN, J. W. Methods of temporarily preserving fresh frozen sections stained with polychrome methylene blue. *Amer. J. Clin. Path.*, 6, 195. 1936.

The tissue coloration produced by polychrome methylene blue usually lasts only 1-2 hrs. in fresh frozen sections. By painting a clear, quick-drying lacquer (*Duco* from E. I. duPont de Nemours Co.) about the edge of the coverslip before the fluid dries, the color can be preserved several days or even weeks. This permits comparison between preserved and fresh tissues.—*V. Warbritton*.

PATRICK, R. "Karo" as a mounting medium. *Science*, 83, 85-6. 1936.

"Karo", a commercial corn syrup containing maltose, dextrose and dextrin, can be used to advantage in mounting algae, pollen grains, and whole insects. Delicate forms require a conc. soln. Advantages: preparations are more permanent than in glycerin jelly; clearing is unnecessary; material can be mounted in it directly from water or lower alcohols; no ringing is necessary; none of the sugars will crystallize out.—*J. A. de Tomasi*.

RUIJTER, J. H. C. Eine Methode zum Umranden von Präparaten. *Zts. wiss. Mikr.*, 51, 374-5. 1935

Ringings of cover glasses with Noyer's lake is annoying when cedar oil is to be cleaned from the slide after observation with an immersion objective. Solvents of the oil are harmful to the lake. The following makes use of a photochemical reaction and works well: 20% gelatin in sat. thymol-water, 100 cc.; 5% $K_2Cr_2O_7$, 10 cc. Keep in the dark. When needed, melt it at 37° C. and distribute with a small brush. When dried in daylight the gelatin is coagulated irreversibly by action of the light, and cannot be removed by any common solvents.—*J. A. de Tomasi*.

SCHMELZER, W. Hecheln und Raspeln, angewandt auf tierische Gewebe. *Zts. wiss. Mikr.*, 51, 516-8. 1935.

Two ways are indicated for teasing and comminuting tissue in order to separate fiber and cell elements. For teasing, it is suggested that a pair of hackle combs be made by securing, for each, 6 medium size sewing needles at the end of a short piece of metal tubing; this end is pressed flat. One of these combs is used to hold the material fast, the other is brought to interlace with the former's prongs and then drawn away from it. The second operation is performed by circular motion, having the material suspended in 1-2 drops of glycerin between 2 pieces of sandpaper.—*J. A. de Tomasi*.

DYES AND THEIR BIOLOGICAL USES

BROOKS, M. M. Mechanism of methylene blue in CO-poisoning. *Proc. Soc. Exp. Biol. & Med.*, 34, 48-9. 1936.

Using spectrophotometric methods on blood from rabbits poisoned with CO and subsequently treated with methylene blue, 0.03% in 0.9% NaCl, 1 cc. per Kg., results indicated that the dye changed CO-hemoglobin into oxyhemoglobin in the blood stream, and not into methemoglobin.—*M. S. Marshall*.

CLARA, M. Über die Diazokuppelungsreaktion zum Nachweis der ortho- und para-Phenole in der histologischen Technik. *Zts. wiss. Mikr.*, 51, 316-37. 1935.

The colorless diazonium salts display the property of combining with phenols, phenoethers, and amino derivatives of aromatic compounds thereby forming colored bodies, the azo dyes. While this reaction can be carried out during the process of staining, the procedure of preparing a suitable diazonium salt is rather cumbersome for the average biologist. The use of already diazotized compounds simplifies the procedure. Experiments with "Nitrosaminrot" and "Nitrazol CF" (Hollborn, Leipzig) show that neither has practical value as they quickly deteriorate. The I. G. Farbenindustrie A. G. has prepared for the author a stabilized product "Echtrotsalz B" which is quite satisfactory. A small amount dissolved in ice-cold water is alkalized with Li_2CO_3 . Sections treated for 30 sec. and rinsed in water stain a light yellow, except the histologic structures containing phenol derivatives, which display a brilliant brown-red tone.—*J. A. de Tomasi.*

FRIEDMAN, M. M. and AUERBACH, O. An improved Congo red test for amyloidosis. *J. Lab. & Clin. Med.*, 21, 93-4. 1935.

By intravenous injection of 10 cc. 1% aq. Congo red, a pathological condition like amyloidosis can be detected, thereby supporting clinical findings. The test is based on the theory that the dye can be recovered quantitatively from the blood serum of normal people; a loss of dye indicates absorption due to amyloidosis. Comparative figures are obtained by the use of the colorimeter, making certain that the serum has not been contaminated by hemolysis. The improvement introduced consists in the use of ethyl alcohol which precipitates the proteins and dissolves the dye, yielding a clear solution suitable for examination.—*J. A. de Tomasi.*

IDE-ROZAS, A. Zwei neue Färbungsmethoden mit Hämatoxylin. *Zts. wiss. Mikr.*, 52, 1-7. 1935.

A report is given on the practical development of a new hematoxylin stain called "Hamatein nach Ide-Rozas", handled by Dr. G. Grubler & Co., Leipzig. It is made up fundamentally of a hematein lake in an AlCl_3 solution. It can be used after all common fixatives, is slow enough so that its staining can be controlled, and does not spoil in solution even in 2 years. A new formula for an iron hematoxylin is as follows: Weigert's hematoxylin, 15 cc.; double dist. glycerin, 5 cc.; iron alum- AlCl_3 solution (5% iron alum and 6% AlCl_3 in dist. water), 5 cc. (The Weigert hematoxylin is prepared by dissolving 1 g. hematoxylin in 10 cc. abs. alcohol and diluting to 100 cc. with dist. water.) Stain 12-24 hr. and differentiate in 2-2.5% iron alum. The staining appears to be much more uniform thruout a section in spite of accidental variations in its thickness.—*J. A. de Tomasi.*

KAWAMURA, R. and YASAKI, T. Ueber eine neue Fettfärbungsmethode. *Zentbl. allg. Path.*, 64, 177-81. 1936. Noch ein Wort zu Unserer Fettfärbungsmethode und über Modification in Herstellung der Stammlösung. *Zentbl. allg. Path.*, 64, 181-3. 1936.

An excellent review is given in these two papers of the present status of fat staining. The following improved Sudan III method is recommended. Grind 4 g. Sudan III (Grubler, Kahlbaum, or Ciba) in a mortar and add to the ground dye 450 cc. of 82% alcohol. Stopper the container loosely and heat slowly until the solution just begins to boil. Filter quickly while hot, set in a refrigerator for 12-24 hr., and filter a second time. This is the *stock solution*. To prepare the *staining solution* take 50 cc. of the stock solution add successive 2 cc. quantities, shaking about 20 times after each addition, until the final volume is 100 cc. Let stand 12-24 hr. at room temp. and filter. The pH should be 5.4 to 5.6.—*H. A. Davenport.*

LIN, F. C. Photodynamic action of methylene blue on diphtheria toxin. *Proc. Soc. Exp. Biol. & Med.*, 33, 337-8. 1935.

Methylene blue, in 0.9% NaCl, in dilution from 1:100 to 1:100,000 with diphtheria toxin, when exposed either to sunlight or to artificial light, brought about marked reduction in toxicity of the toxin.—*M. S. Marshall.*

LISON, L. **Sur le mécanisme et la signification de la coloration des lipides par le bleu de Nil.** *Bull. d'Histol. Appl.*, 12, 279-89. 1935.

It is understood that Nile blue sulfate can hydrolize, but only in solutions of high dilution; the greater the concentration the less the hydrolysis, in accordance with the mass law. From a dilute solution any fat solvent will extract an orange-red fraction called here "Nile red". It is found that the free base of the dye (prepared by alkalinizing, extracting with toluene, drying and redissolving in alcohol) does not stain fatty materials. The Nile red instead is the fraction responsible for the pink stain imparted to many substances. Commercial Nile blue always contains Nile red beside the true blue. Both of them stain fatty substances without displaying a definite specificity.—*J. A. de Tomasi.*

MICHAELIS, L. and SMYTHE, C. V. **Influence of certain dye-stuffs on fermentation and respiration of yeast extract.** *Proc. Soc. Exp. Biol. & Med.*, 33, 127-9. 1935.

To an actively reducing yeast extract were added various dyes, including 1-naphthol 2-sulfonate indophenol, thionin, pyocyanin, indigo disulfonate, and pheno-safranin. The result was frequently an initial acceleration of fermentation followed by inhibition, but this inhibition did not correlate with oxygen consumption. It was irreversible and was due to destruction of enzymes.—*M. S. Marshall.*

STOWE, W. P. and DELPRAT, G. D. **The rose bengal test of liver function: photelometric method.** *J. Lab. & Clin. Med.*, 20, 1297-8. 1935.

Rose bengal in 1% normal saline solution is injected intravenously for the comparative test of blood in a colorimeter. Correct reading is secured by means of a photoelectric cell, which eliminates the personal equation error. Methods of operation and calculation of results are given, but as they have no direct bearing on microtechnic, are not detailed here.—*J. A. de Tomasi.*

ANIMAL MICROTECHNIC

BECKER, W. S., PRAVER, L. L. and THATCHER, H. **An improved (paraffin section) method for the Dopa reaction.** *Arch. Dermat. and Syphilol.*, 31, 190-5. 1935.

A paraffin method is given for the Dopa reaction which is superior to the frozen section method. Immerse fresh tissue 3-5 mm. thick in a solution of di-oxy-phenylalanine "prepared according to the simplified technic of Laidlaw and Blackberg" (*Amer. J. Path.*, 8, 491, 1932). Change the solution after ½ hr. The reaction of the solutions varies from pH 7.35 to 7.8. Incubate the solution containing the tissue at 37° C. for 12-15 hr. During this time the tissue and the solution turn black. Fix the block in Bouin's solution "according to the method of Masson" for 48-72 hr. Wash and dehydrate in alcohol; pass thru toluene; embed in paraffin containing 5% beeswax. Cut sections 5-6 μ and follow the usual technic down to water. Wash in running water for 1 hr. The trinitrophenol washes out and the collagen appears gray. For a simple counterstain brazilin may be used. For cytological study use the Masson trichrome stain (iron hematoxylin, acid fuchsin-ponceau de xyldine and anilin blue) as a counterstain. Identification of the Dopa-positive cells is enhanced by a light staining of the protoplasm with the acid fuchsin-ponceau de xyldine mixture.—*M. Lois Calhoun.*

BLAIR, D. M. and DAVIES, F. **Observations on the conducting system of the heart.** *J. Anat.*, 69, 303. 1935.

A method is described for demonstrating the conducting and nervous systems of the heart. When large blocks of tissue are stained *in toto* by this technic, sections show well impregnated nerve fibers and clearly differentiated muscle fibers. Shrinkage is minimal.

Fix in 10% formalin (4% formaldehyde neutralized with ammonia) for 10 days, or longer. Place 48 hr. in: 10% formalin, 95 cc.; NH_4OH , 5 cc. Wash 48 hr. in running tap water. Wash 3 hr. in many changes of dist. water. Place 24 hr. in pyridin. Wash 4 days in many changes of dist. water. The tissue is then handled in the dark until dehydration. Put 4 days in 2% AgNO_3 in an incubator at 35° C. Wash 3 hr. in many changes of dist. water. Place 24 hr. in: pyrogallie acid, 4 g.; 5% formalin, 100 cc. Wash 1 hr. in dist. water. Transfer to 50% alcohol, dehydrate, clear in benzol or chloroform, and embed in paraffin.

To insure best results, the specified number of hours for fixing and staining should be rigidly adhered to. Washing should be prolonged if the reagents are not thoroly removed.—*H. D. Reed.*

DE GALANTHA, E. A new stain for connective tissue, mucin and allied substances. *Amer. J. Clin. Path.*, **6**, 196-7. 1936.

Orange S (E. I. duPont de Nemours & Co.) makes a good contrast stain in various combinations. The stock solution contains 1 g. orange S in 200 cc. 95% alcohol and 10 cc. glacial acetic acid. For use dilute 50 cc. with 25 cc. abs. alcohol. To stain connective tissue and mucin, treat formalin preserved sections as follows: Stain 30 min. in sat. aq. solution picric acid, 75 cc., plus glacial acetic acid, 5 cc.; wash in water; stain 10 min. in alum hematoxylin (volume and concentration not specified) plus 10 drops orange S staining solution; wash in water 10 min.; stain 20 min. in alum lake carmine made up fresh each time as follows: heat 2 g. alum carmine and 0.5 g. Al Cl₃ slowly in 20 cc. dist. water, until deep red, add 100 cc. 95% alcohol and 80 cc. dist. water, filter. Wash quickly in water; stain 5 min. in a mixture of 200 cc. sat. aq. solution of picric acid and 20 cc. 1% aq. indigo carmine; wash quickly in water; dehydrate in 95% methyl alcohol, and in acetone; clear in xylene; mount with Canada balsam. The colors are: mucin, brilliant red; cartilage and hyaline substances, bluish red; fibrillar and collagenic connective tissue, grass green; smooth muscle, olive green; striated muscle, yellow. For amyloid, treat sections as follows: Harris hematoxylin, 5 min.; steaming carbol fuchsin, 5 min.; wash in tap water; decolorize in acid alcohol (1% HCl in 70% alcohol); wash 10 min. in running tap water; stain in picro-indigo-carmin solution, 5 min. and in orange S solution, 1 min.; decolorize in abs. alcohol; wash in abs. alcohol; clear in carboyl-xylene, and xylene; mount in Canada balsam. Amyloid stains greenish gray; connective tissue, green; erythrocytes, yellow; and nuclei, reddish brown.—*V. Warbritton.*

GALL, E. A. The technic and application of supravital staining. *J. Lab. & Clin. Med.*, **20**, 1276-93. 1935.

A thoroly critical digest of the theory and practical use of vital staining in the diagnosis and classification of blood elements. The cell forms of normal and pathological blood are described in detail, and their characteristics are mapped out in a reference chart. All routine laboratory steps of the neutral red-Janus green technic are given. It is argued that the supravital method cannot be relied upon to the exclusion of the fixed smear technic.—*J. A. de Tomasi.*

GRAY, J. H. Preliminary note on the mast cells of the human pituitary and of the mammalian pituitary in general. *J. Anat.*, **69**, 153. 1935.

The pituitary was fixed by the intracardiac injection of 10% neutral formalin in normal saline during ether anesthesia. If alcoholic staining solutions were to be used, the sections were neutralized in acid or alkaline alcohol during differentiation. If the staining solutions were made up with non-alcoholic solvents, the sections were neutralized in acid or alkaline baths previous to staining.—*H. D. Reed.*

HEIDERMANNS, C. and WURMBACH, H. Eine Methode zum histochemischen Nachweis geballten Phosphats im Gewebe. *Zts. wiss. Mikr.*, **51**, 375-8. 1935.

So far the histochemical detection of phosphates has limited itself to the qualitative determination of the cation, mostly Ca. Now a dark red color reaction is proposed, specific for the P anion. It is based upon the property of uranyl salts to develop a ppt. with PO₄ insoluble in acetic acid, soluble in dilute mineral acids. The following schedule is suggested: from water, soak sections 1½-2 hr. in 1% uranyl sulfate; rinse in water; dip quickly in 1% HNO₃; rinse in water; treat 20 min. in 5% K₄Fe(CN)₆; rinse in water; dip 2-5 min. in 5% HNO₃; rinse repeatedly in water; counterstain 10 min. with thionin; dehydrate, clear and mount. Ossification centers appear a dark red-brown due to PO₄; cartilage, purple-red; connective tissue, blue; blood cells, green.—*J. A. de Tomasi.*

HIRAKO, G. Beiträge zur wissenschaftlichen Anatomie des Nervensystems. *Folia Anat. Jap.*, 13, 561-6. 1935.

The author tries out a number of methods in the study of the Purkinje cells of the cerebellum including: Nissl's, Golgi's, Bielschowsky's, Cajal's, Dogiel's vital stain, Carnoy hematoxylin, Müller-Retzius'; but by far the most satisfactory is the Weigert-Pal hematoxylin medullar stain. (Details of the methods not given.) It is not only selective for the Purkinje cells, but surpasses any of the others in developing a complete picture of even their finest outer branches.—*J. A. de Tomasi.*

KATO, K. Monophyletic scheme of blood cell formation for clinical and laboratory reference. *J. Lab. & Clin. Med.*, 20, 1243-1252. 1935.

While no pretense is made of formulating a new theory of hemocytogenesis, the blood cells are once more reclassified on the basis of their clinical significance. The purpose is to systematize the nomenclature, to redefine the various cell types and to arrange them genetically in accordance to the monophyletic neounitarian theory. Staining is carried out by means of Pappenheim's panoptic combination (May Grunwald stain followed by dilute Giemsa) The scheme of classification is discussed at length and summarized in a 3-color chart commendable for its fine topographical appearance.—*J. A. de Tomasi.*

MARZA, V. D. The formation of the hen's egg. V. Histochemistry of yolk formation. Proteins. *Quart. J. Mic. Sci.*, 78, 191. 1935.

This paper contains a standardization of Unna's method for the detection of acid proteins in developing eggs. The ovaries are fixed in either 96% alcohol or in acetic sublimate and embedded in paraffin.

Three series of sections are made: a) the first is untreated and is used to detect the total acid protein; b) the second is treated, after paraffin removal, with sterile water in an incubator at 40° C. for 24 hrs.; this dissolves out the cytoseres and pseudo-globulins; c) the third is treated, after paraffin removal, with sterile 2% NaCl in an incubator at 40° C. for 24 hrs.; this dissolves out the globulins as well as the cytoseres leaving only the nucleoproteins.

The sections must all be the same thickness. A clamp arrangement is devised for holding slides so that all three series can be treated at once with the stain and dehydrating reagents.

The sections are stained for 20 min. in methyl-green-pyronin: methyl green (purified by shaking with chloroform), 0.15 g.; pyronin, 0.25 g.; 96% alcohol, 2.5 cc.; 0.5% carboic acid, 100 cc. Wash rapidly in water for 1 or 2 sec. Pass thru 4 changes of 96% alcohol, 5 sec. in each. Clear in xylol and mount in Canada balsam.

Four staining intensities for pyronin can be distinguished: pale pink, light red, red, and deep red. The relative amount of acid protein can then be roughly ascertained by comparing the differently treated sections, the more acid protein the deeper red the color.—*H. D. Reed.*

MIYAMOTO, K., YAMADA, K. and SHISHIDO, S. Dunkelfeld-Untersuchungen an überlebenden Triton-Erythrozyten. I. Vorläufige Mitteilung. Über den Einfluss der H-ionenkonzentration auf die Triton-Erythrozyten. *Folia Anat. Jap.*, 13, 509-12. 1935.

This darkfield investigation is part of an extended study on the influence of H-ion concentration on erythrocytes. In the blood of Triton it has been claimed that below pH 7.0 granules and rodlets appear while at a pH higher than 7.2 there is a development of a net- or mesh-like structure. The darkfield technic is applied to investigate this claim. The production of independent granules is verified and might be explained by the polarity of the erythrocytes and the incidental "gelatinization" effect of the supravital dyes, rather than by the dispersion changes of the plasma sols due to varying the H-ion concentration.—*J. A. de Tomasi.*

PASTEELS, J. and LEONARD, G. Sur la détection du glycogène dans les coupes histologiques. *Bull. d'Histol. Appl.*, 12, 293-9. 1935.

This is a study of the detection of glycogen as well as of the effect of various fixatives upon trout and amphibian eggs, also various liver and muscle tissues. Altho its specificity is not the highest. Bauer's stain (formula not given) is the most reliable provided a control is run covering salivary digestion and Lugol tests. Embedding is preferably carried out by transferring the material from the fixing bath to dioxan containing a little CaCl_2 , and after 20-30 min. passing to dioxan-paraffin, and paraffin. A large number of fixatives were studied: abs. alcohol, alcohol-formol, dextrose-formol, Sauer-Carnoy's, Zenker's, Bouin's, and Bouin-Allen's. As far as preserving glycogen is concerned, the most reliable proved to be those in aq. solution, Bouin-Allen's being the best. A still better fixative for embryonic tissues is the following: sat. picric acid soln. in dioxan about 35%, 85% by volume; formalin, 10%; glacial acetic acid, 5%. Fixation is attained in 1 hr. Other experiments show also that picric acid is preferable to various combinations of plain alcohols and formalin.—*J. A. de Tomasi.*

PICK, J. Einige Vitalfärbungen am Frosch mit neuen fluoreszierenden Farbstoffen. *Zts. wiss. Mikr.*, 51, 338-51. 1935.

Generally speaking, vital and intravital stains make use of dyes whose color is perceived by transmitted illumination. There is also a group of dyes which can be seen by fluorescence thru irradiation with ultraviolet light. This group is indicated here with the generic name of "Fluorochrome" and is studied at length with respect to physical and chemical properties of its individual members. Various types of tissue from the frog serve as experimental material. It is found that dyes appearing red in transmitted light (geranin, rhodamine, eosin, magdala red, erythrosin) are not of practical value in intravital fluorescence work. The following (with their fluorescence) are more promising: primulin yellow, blue-violet; primulin, bluish violet; thiazol yellow, blue-violet; thioflavin, blue-violet; brilliant phosphin G extra, orange; rheonin A, greenish; berberin sulfate, yellowish; and styryl-chinolin, greenish. With these there should be included two plant pigments: chelidonium extract, light yellow; and extractum radices rhei, bluish.—*J. A. de Tomasi.*

PICKWORTH, F. A. A new method of study of the brain capillaries and its application to the regional localization of mental disorder. *J. Anat.*, 69, 62. 1934.

In this method the blood is stained *in situ*, thereby obviating the hazards of injecting dyes to make the blood vessels visible. Hemoglobin forms a blue-black pigment with benzidine-nitroprusside mixture in the presence of an oxidizing agent. This pigment is insoluble in organic liquids. The method can be used for other tissues.

The brain is fixed *in situ*. This is done by cutting thru the skull 1 in. above the auditory meatus and 1 in. above the eyebrow, and then, before disturbing the vault, cutting thru all tissues and brain with a fine hacksaw. The part in the vault is fixed *in situ*, and the remainder is also fixed *in situ* by attaching a 4 in. section of a 6 in. inner tube to form a basin. Fix 24 hrs. in formal hypertonic saline: 40% (commercial) formalin, 100 cc.; NaCl , 20 g.; water, 1000 cc. The brain is then cut into slices not more than 1 cm. thick and further fixed in fresh formol hypertonic saline for 2 days. Wash in water for several hours. Soak slices for 2 days in gum phenol solution: gum arabic, coarse powder, 500 g.; water, 2000 cc.; phenol, 20 g.; warm and filter thru wool and then by pressure thru a Leitz bacterial filter. Cut sections at 250 μ (or less) with freezing microtome. Wash in running water for 2 hrs. to remove gum and formalin. Stain sections 1 hr. in a shaking machine at 37° C. in the following benzidine-nitroprusside mixture: Sodium nitroprusside, 0.1 g.; 0.5% benzidine in 2% acetic acid, 25 cc.; dist. water, 75 cc.; dissolve the nitroprusside in 20 cc. water and add the benzidine, make up to 100 cc. by adding dist. water, and filter. The solution must be prepared directly before using. Rinse quickly in water. Place slices in weak hydrogen peroxide (2 cc. in 400 cc. dist. water) in a shaking machine at 37° C., shaking gently for 1 hr. Wash in water, dehydrate, clear in xylol, and mount in balsam.

The tissues should be clear and transparent. The blood vessels are seen as well-defined jet-black threads. If the balsam is acid-free the sections will keep well for over 6 months.—*H. D. Reed.*

ROBERTS, W. J. Recherche de l'or dans les tissus animaux par la méthode du développement physique. *Bull. d'Histol. Appl.*, 12, 344-61. 1935.

This is a study of the sensitivity of the so-called method of "physical development" for the detection of gold in animal tissue sections. The method is based on the precipitation of Ag^+ on the gold from a combination of AgNO_3 , hydroquinone and citric acid, balanced in such a way that no Ag will separate unless Au is present. No metal should be used for fixation; formol or Bouin's fixatives are satisfactory. The technic can be used for both frozen and paraffin sections. The latter are secured to the slide with dist. water and are cleared in 3 baths of xylene. The schedule follows: Solution A: AgNO_3 , 2 g.; 10% gum arabic, 100 cc.; prepare freshly and keep in the dark. Solution B: hydroquinone, 1 g.; 10% gum arabic, 100 cc. Hold 24 hrs. before use. Fix material in 20% formol, wash thoroughly in water, gather sections in dist. water; mix 2 cc. of Sol. A with 2 cc. of Sol. B. and add 1-3 drops of 5% citric acid. Stir 30 sec. and treat the sections 5-10 min. Transfer for a few minutes to 5% $\text{Na}_2\text{S}_2\text{O}_3$. Wash thoroughly and mount, with or without counterstain.—*J. A. de Tomasi.*

SCHMIDT, W. J. Dichroitische Gold- und Silberfärbung des Aussengliedes der Sehzellen vom Frosch. *Zts. wiss. Mikr.*, 52, 8-23. 1935.

The idea of using heavy metal impregnation is applied to the study of retina sections. The method relies upon the precipitation of Ag as well as Au on the cellular elements and on the use of polarized light for their examination. By rotating the Nicol's prisms a double color effect is obtained which accounts for the designation "dichroic stain" given the method. Proceed as follows: Spread sections fixed in Zenker fluid (without acetic acid) upon a glass slide. Run down to water. Soak 30 min. in 0.25-0.5% AuCl_3 or 1% aq. AgNO_3 solution. Transfer to 100 cc. dist. water plus 1-2 drops hydrazine hydrate. In a fraction of a minute the reduction of the metal is completed. Wash thoroughly in dist. water, run up to xylene and mount in Canada balsam. The dichroism is limited to the outer segment of cones and rods of the retina. With gold, the color produced is a dirty wine red when the cellular elements lie parallel to the vibration plane of the polarizer, and blue-gray or blue-black when they are perpendicular to it. With silver, the colors are, respectively, greenish-yellow and rust-brown or black. The balsam also acts as a reducing agent on such preparations and can be substituted for the hydrazine hydrate by simply eliminating the latter from the schedule. The reduction is ordinarily completed in a few hours, rarely requiring 1-2 days. With gold, the colors are, respectively, brilliant wine red and dark blue. All these color effects are reproduced in a trichrome plate appended to the article.—*J. A. de Tomasi.*

SOKOLANSKY, G. Versuchsergebnisse einer Färbung von Myelin-Nervenfaseren in Gefrierschnitten nach eigener Methode. *Zts. wiss. Mikr.*, 51, 378-83. 1935.

For the detection of myelin nerve fibers in frozen sections the following methods prove quite satisfactory: (1) For general orientation only: Fix 2-3 days in formalin; cut 20-30 μ frozen sections; soak 24-72 hr. in concentrated $\text{K}_2\text{Cr}_2\text{O}_7$ at 37-40° C.; rinse in water and stain 5-20 hr. in Kultschitzky's hematoxylin at the same temperature; transfer for 2-3 min. to the Müller's solution. Rinse in water; differentiate according to Pal (oxalic acid and 2% Na_2SO_4); abs. alcohol, carbol-xylene, and Canada balsam. (2) For finer study of details: Cut 5-8 μ frozen sections and soak 24 hr. in concentrated $\text{K}_2\text{Cr}_2\text{O}_7$ at 37-40° C.; stain 4-6 hr. in Kultschitzky's hematoxylin at 50-60° C. or 48 hr. at 40° C. Follow schedule (1) to the end. The sections ought to be kept separated in the bichromate as well as in the staining solution. At times, 10 min. in 70-80% alcohol before the bichromate prevents uneven "chromation"; after this, the yellow color must be washed away as much as possible by a water rinse.—*J. A. de Tomasi.*

THOMAS, J. A. and LAVOLLAY, J. Une réaction histochemique du fer à la 8-hydroxyquinoléine. *Bull. d'Histol. Appl.*, 12, 400-2. 1935.

The reaction with 8-hydroxyquinoline, resulting in the production of a green-black compound, is considered specific for Fe^{+++} and can be employed for the detection and location of iron salts in animal tissues. It is not interfered with by fixatives like alcohol, trichloroacetic acid, neutral formol, and Bouin's. The compound formed is soluble in the common organic solvents. The technic is as follows: Dissolve 2.5 g. 8-hydroxyquinoline (orthoxyquinoline) in 4 cc pure acetic acid, add dist. water up to 100 cc. and filter. Place a few drops of the reagent on the sections, well fixed and washed in dist. water. After 5-15 min. drain and replace with 1 drop of conc. NH_4OH in dist. water. Wash away the resulting precipitate with dist. water. Stain the nuclei with lithium carmine. Mount in neutral dist. water or after partial drying, dehydrate in terpinol and mount in vaseline oil. Examine promptly as the intensity of the stain decreases appreciably within the first 24 hr.—J. A. de Tomas.

WILLIAMS, B. G. R. Observations on intensifying the metachromatic properties of cresylecht violet. *J. Lab. & Clin. Med.*, 20, 1185-7. 1935.

In the development of a new method for preparing a polychrome staining solution from cresylecht violet it is found that, contrary to expectation, alkalization is practicable. A good dye must be used and great care should be exercised in the manipulation. The method follows: Mix cresylecht violet (National Aniline & Chem. Co.) 1 g.; dry K_2CO_3 , 1 g.; formalin (U. S. P.), 5 ml.; dist. water, 95 ml. Shake at intervals for 30 min. Add 3 cc. glacial acetic acid in small amounts while agitating. Do not cork; shake at intervals for 30 min. Filter and add 5 ml. 99% isopropyl alcohol. The solution is used in surgical pathology and for rapid examination of tissues (stain the wet sections directly for about 6 sec., then wash them in tap water). The color reactions are: all nuclei and muscle cytoplasm, blue; other cytoplasm and fibrils, pink; fat cells, yellow.—J. A. de Tomas.

PLANT MICROTECHNIC

KISSER, J. Bemerkungen zum Einschluss in Glycerin-Gelatine. *Zts. wiss. Mikr.*, 51, 372-4. 1935.

There are several disadvantages in the use of glycerin-gelatin as a mounting medium for plant sections. It very easily takes up impurities which then appear as contaminants in the mount and are difficult to eliminate. Air bubbles are held fast, and the mounting medium shows a tendency to dry up and shrink. In most cases it is believed that the medium as well as the sections at the time of mounting carry too much water. A new formula for glycerin-gelatin which proved satisfactory on a pliable substrate (celluloid sheets) is the following: gelatin, 50 g.; water, 175 cc.; glycerin, 150 cc.; phenol, 2-3 g. The sections must be transferred to the mount directly from glycerin instead of from water. In the case of mounts on glass slides, the cover glass is not absolutely necessary; if used, ringing can be eliminated.—J. A. de Tomas

MICROÖRGANISMS

GRAF, W. Untersuchungen über die Verwertbarkeit der Farbstoffreduktion zur Differentialdiagnose in der Salmonellagruppe. *Zentbl. Bakt., I Abt. Orig.*, 135, 377-85. 1935.

Knowledge of the theory of redox potentials is of paramount value in the choice of proper dye-stuffs to be used in bacteriological diagnostic work. Several dyes are suitable for use in nutrient media, and their color reduction is correlated to different serologic groups of the *Salmonella* genus. To make sure of uniform material Merck's broth (Standard I) at pH 7.4 is used thruout. Any sugar that may be present is taken out by yeast or *B. coli* fermentation, and semisolid media are made up with 0.1% agar. The color of the media prevents utilization of yellow and brown dyes. Concentration is a delicate item on account of the toxicity factor; for this reason it is best to add the dyes to the liquid cultures after incubation for 15-20 hr. To prevent reoxidation, a layer of paraffin oil is poured over the cultures.

Rosindulin GG (Hollborn), used in the same concentration as was used by Ishiyama for chrocein (*Zentbl. Bakt., I Abt. Orig.*, 111, 24, 1929), is reduced within 24-36 hr. by paratyphoid B strains but not before 3 days by paratyphoid A strains. Indigo carmine, Janus green and neutral red are not satisfactory. While 50 strains of Breslau and 50 of Schottmüller could not be differentiated by 23 dyes studied, "gaslos" varieties of the two groups could be differentiated by Janus green and neutral red (but not beyond pH 7.5). Furthermore, R and S dissociation is correlated with changes in the degree of reduction of the indicators.—*J. A. de Tomasi.*

KAUFFMANN, F. Weitere Erfahrungen mit dem Anreicherungsverfahren für Salmonellabacillen. Zts. Hyg. u. Infektionskr., 117, 26. 1936.

As enrichment media for bacteria of the *Salmonella* group, the following were tried:

1. Modified Drigalski agar: To 1000 cc. of 2% agar in bouillon at pH 7.4-7.6 add: lactose, 15 g. 1% crystal violet, 5 cc; $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, 1 g. Litmus solution (Kahlbaum-Schering 15%).

2. Brilliant green agar (modification of Kristensen, Lester, and Jürgens' nutrient medium): Liebig's meat extract, 0.5%; peptone (Riedel), 1%; NaCl, 0.5%; lactose, 1.5%; phenol red solution, (0.1 N NaOH, 40 cc., distilled water, 460 cc.; phenol red, 1 g.) 0.5% brilliant green solution, 2.5 cc. per liter; agar, 2.5%; pH 7.0-7.2.

3. Combined "Enrichment" nutrient medium (Kauffmann):

A. Preparation of tetrathionate bouillon: Sterile bouillon, 90 cc.; sterilized CaCO_3 , 5 g.; 5% $\text{Na}_2\text{S}_2\text{O}_3$ in dist. water, 10 cc.; iodine solution (I_2 , 20 g.; KI, 25 g.; dist. water to 100 cc.), 2 cc. This mixture, unheated, is added to the following solutions:

B. 1:1000 brilliant green in dist. water, 1 cc. to 100 cc. of tetrathionate bouillon.

C. Sterile bovine bile, 5%.

The combined solutions are shaken until the CaCO_3 is evenly distributed, then poured into sterile test tubes to a depth of 7-8 cm. The medium is then ready for use, and may be kept for a long time before using.

Inoculation must be heavy, either by adding masses of feces directly or by adding 0.5 cc. of a fluid extract of the feces. The cultures are incubated at 37° for 16-20 hr.

By using the combined "Enrichment" nutrient medium, an increase was found in positive tests for *Salmonella* bacilli in comparison with the results obtained with modified Drigalski agar or brilliant green agar.—*H. D. Reed.*

MERLING-EISENBERG, K. B. Internal structure of bacterial cells. Brit. J. Exp. Path., 16, 388-93. 1935.

The author gives two methods for demonstrating the intracellular structure of bacteria. (1) A thin film of the culture is allowed to dry on a slide 0.9 mm. thick and covered with a drop of oil of high refractive index (ordinary immersion oil is sufficient in most cases) and a cover glass 0.1 mm. thick. Bacteria will remain alive for a period varying from some minutes to several weeks according to the organism employed, and may be regarded as normal during the time of examination. View the preparation with dark ground illumination. An effect especially useful in photomicrography may be brought about by the use of an E. Leitz-Wetzlar condenser (N.A. 1.40) in conjunction with an apochromat objective (N.A. 1.40) stopped down to 1.37. (2) With the second method the bacteria are observed in their natural aq. media. This is possible by arranging a one-sided oblique illumination by fitting an azimuth diaphragm to the dark-ground condenser.—*M. Lois Calhoun.*

NIETO, D. Über die Bedingungen des Spirochätennachweises in einzelnen Schnitten und ein bisher zu diesem Zweck noch nicht benutztes Prinzip. Zts. wiss. Mikr., 51, 528-31. 1935.

The principle underlying a new spirochaete stain in individual sections (see Klin. Woch., 1975-6, 1933) is explained as follows: The sections are transferred to a mixture of AgNO_3 and developed as usual; but, instead of a protective colloid

(e.g., gelatin), an organic acid (acetic or citric) is used. Upon adding hydroquinone or pyrogalllic acid a retardation of the development is brought about similar to that secured by means of a protective colloid.—*J. A. de Tomasi.*

STARK, C. N. and CURTIS, L. R. Evaluation of certain media for the detection of colon organisms in milk. *Amer. J. Pub. Health*, 26, 354-6. 1936.

The ideal medium for detection of colon organisms in milk should permit growth and gas production by small numbers of *Escherichia-Aerobacter* organisms, and inhibit the growth of those lactose-fermenting gas-producing bacteria not belonging to this group. Pure culture tests show that crystal violet, Dominick-Lauter, gentian violet, and brilliant green bile broths are not satisfactory for this purpose, but that formate ricinoleate broth does fulfill the requirements of an ideal medium.—*M. W. Jennison.*

SWARTZ, J. H. and CONANT, F. Direct microscopic examination of the skin. A method for the determination of the presence of fungi. *Arch. Dermat. and Syphilol.*, 33, 291-305. 1936.

A simple technic is presented for the microscopic examination of cutaneous scales and the determination of the presence or absence of fungi. Clear the scales in 5% solution of KOH. Transfer to a watch crystal and wash with water for 2-3 min. Heat the scales gently in a drop of the following solution on a slide: lactic acid, 1 cc.; phenol crystals, 1 g.; glycerin, 2 cc.; dist. water, 1 cc.; add 0.5% cotton blue (C₄B Poirrier). The epidermal cells become a light blue while the granular protoplasm of the fungus stains heavily. If the fungus stains slightly, try staining with 1% cotton blue in 70% alcohol instead of the above stain. Mount in clear lactophenol and press out under a cover slip. For permanent preparations clean off excess lactophenol around the cover slip and seal with Noyer's cement.

Another method of obtaining permanent preparations is to mount the stained scales in chloral hydrate and acacia: dist. water, 5 cc.; chloral hydrate, 50 g.; glycerin, 20 cc.; acacia, 30 g. The permanency has been tested only for 5 months.—*M. Lois Calhoun.*

TURNER, A. W. Pleuro-pneumonia contagiosa Boum: The staining of the causal organism in the specific lesions. *Austral. J. Exp. Biol. Med. Sci.*, Sept., 149. 1935.

The Nocard and Roux organism of pleuro-pneumonia in cattle can be demonstrated easily in sections of pathological tissues by a variety of staining methods providing it is suitably fixed. Formol-saline, formol-Muller, or Regaud fixatives are contra-indicated. Suitable fixatives are: the mercuric chloride group (especially Zenker), Bouin (picro-formol-acetic), abs. alcohol, and Carnoy. The recommended technic for routine purposes is fixation in Bouin's solution followed by the staining of paraffin sections with Mallory's phosphotungstic acid hematoxylin for 18-24 hr. Blot nearly dry without washing, and quickly dehydrate with abs. alcohol. Clear in xylol, mount in balsam.

The organisms stain a deep blue and appear distinctly even under the low power as thick tangled masses of the mycelial phase. The nuclei stain blue, protoplasm and muscle various shades of blue, mucin reddish yellow, cartilage deep rose red, fibrin deep blue, and erythrocytes steel blue.—*Elizabeth Bachels.*

ERRATUM SLIP

The following error occurs in the paper by McWhorter and Weier in the July number (pp. 107-17):

The cuts for Fig. 4 (p. 112) and Fig. 6 (p. 115) are transposed, so that the legend under the latter refers to the former, and vice versa.

STAIN TECHNOLOGY

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IMPROVING THE TECHNIC OF THE FEULGEN STAIN

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ABSTRACT.—A study has been made of the Feulgen stain, in which the staining fluid is a decolorized basic fuchsin. Particular attention has been given to the variation in behavior of different fuchsin samples, the reagent to be employed in decolorizing the dye, the acidity of solutions, and the value of several counterstains. A modified procedure is suggested, the details of which are given in the paper. The principle modifications of earlier procedures which are recommended are as follows: the use of a specially purified pararosanilin as a dye; the employment of $K_2S_2O_8$ instead of $NaHSO_3$ as a decolorizing agent; and counterstaining with fast green in the case of plant tissue or with orange G for animal material.

INTRODUCTION

The Feulgen procedure, which is now coming into prominence as a cytological stain, was originally developed by Feulgen and Rossenbeck (1924) as a microchemical test. It was designed to distinguish that type of nucleic acid found in chromatin (which contains a hexose radical) from other similar acids which contain pentose instead of hexose groups. The Feulgen reaction is based upon the use of a reduced (colorless) form of basic fuchsin, which develops a specific purple color in contact with an aldehyde.

As the ending "al" is used in the Geneva chemical terminology to indicate a compound of aldehydic nature, it is quite natural that Feulgen should have called his stain a "Nuclearreaktion." It is generally assumed, as many have stated, that it is quite a sharp reaction based upon the liberation of free aldehyde groups by hydrolysis of the thymonucleic acid from the chromatin. While its sharpness and significance may still be open to discussion, no doubt the high degree of specificity and the clean yet delicate differentiation it affords are of great appeal to the biomicroscopist. In spite of such advantages, however, it has never been as widely used as many other

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nuclear stains that do not possess its inherent good qualities. Accordingly, an investigation has been carried on by the Stain Commission in hopes of simplifying and standardizing the procedure so as to bring it into more general use. The present paper deals with the staining technic; a paper by Scanlan and Melin, to appear in the next issue, will discuss the question of the proper dye to employ.

THE METHOD AND ITS MODIFICATIONS

A survey of the literature shows a great many variations from the original method as given by Feulgen and Rossenbeck (1924). The original directions included, for instance, a reagent (1% dimethylcyclohexandione, a cycloparaffin derivative) which has since been dropped by the majority of those who worked with the stain. The purpose of adding this to the descending alcohols was to bind the aldehydes always present in alcohol and prevent the oxidizing effect of atmospheric oxygen on them. These compounds were supposed to be harmful and detrimental to the action of the stain. The original procedure, among other things, included also a combination of steps for the hydrolization of the thymonucleic acid with warm HCl. Furthermore, the preparation of the stain solution and the rinsing bath to follow it called for sodium bisulfite (i.e., acid sodium sulfite, NaHSO_3) as a source of SO_2 for the reduction of the dye. Altho the general scheme of the original method has been followed by later workers, nevertheless, a great number of variations have been introduced which at times do not seem to warrant the innovation. Such changes or new interpretations involve timing, concentration of the reagents, and various other factors. This becomes evident by consulting any of the outstanding papers on the Feulgen stain of the last few years. See, for instance, the work of Westbrook (1930), Boas and Biechele (1932), Milowidow (1933), and Margolena (1932). The last-named author, for example, gives the following schedule:

Preparation of fuchsin solution: Dissolve 1 g. basic fuchsin by bringing to boiling in 200 cc. dist. water. Cool to 50° C., filter, add 20 cc. 0.5 N HCl. Cool to 25° C., add 1 g. sodium bisulfite.

Staining: Run down to water; 0.5 N HCl, 2 min. or more; 0.5 N HCl at 60° C., 2 min. or more; rinse in cold HCl; dip in water. Stain in fuchsin, 1-3 hr.; wash in 3 baths of the following: 200 cc. dist. water, 10 cc. 0.5 N HCl, 10 cc. 10% sodium bisulfite. Rinse in dist. water, run up thru the alcohols, counterstain with any of the usual counterstains, dehydrate, clear and mount.

In the light of these directions, a brief discussion follows of the major features of the method, as preliminary to an explanation of those changes now proposed as experimentally sound and distinctly advisable.

EXPERIMENTAL

Preparation of Fuchsin Solution: The purpose of dissolving the dye in a solution of the kind in question is to produce a stain temporarily "bleached" or colorless which will work selectively and be fixed specifically by the nuclear matter. It is obvious that the theory is very much the same as in the case of Schiff's reagent: a solution of magenta (fuchsin) is barely decolorized with SO_2 ; if an aldehyde is present in any solution to which this reagent is added, a bright pink color soon appears. Ketones do not give the same reaction.

TABLE 1. DATA CONCERNING DECOLORIZED SOLUTIONS OF VARIOUS FUCHSIN SAMPLES

Sample No.	pH	Age	Color of Solution		Staining
			Fresh	Aged as in Column 3	
EXPERIMENTAL SAMPLES					
1	1.63	8 months	Light straw	Yellow	Excellent
2	1.69	8 months	Yellow brown	Red brown	Quite good
3	1.70	8 months	Pink brown	Deep pink	Not good
4	1.75	8 months	Pink brown	Deep red pink	Too dark
5	1.52	8 months	Purplish pink	Deep red pink	Not good
6	1.62	8 months	Straw yellow	Deep red pink	Excellent
7	1.75	8 months	Light straw	Red pink	Satisfactory
8	1.35	4 months	Light straw	Red pink	Satisfactory
9	1.40	4 months	Light straw	Red pink	Satisfactory
10	1.33	4 months	Light straw	Red pink	Satisfactory
COMMERCIAL SAMPLES					
11	1.33	6 months	Gold yellow	Red pink	Mediocre
12	1.58	4 months	Deep orange	Red pink	Satisfactory
13	1.42	4 months	Light straw	Red pink	Excellent
14	1.45	4 months	Yellow straw	Red pink	Satisfactory

There have been tested in this laboratory nearly 80 samples of basic fuchsin, some commercial samples submitted by stain companies, others experimental products from the Industrial Farm Products Research Division at Washington. The object was to learn which one of these samples would decolorize satisfactorily to a light yellow color, would give the least precipitate and a correct tinting of the chromatin in the Feulgen technic. It was found that only a few of the commercial samples were altogether satisfactory and that products from the same source would vary greatly from time to time. Commercial basic fuchsins vary greatly in composition (Conn, et al., 1936, pp. 99 to 103), so that it is difficult to be certain what any given sample may be. On the other hand, after much work

at Washington, the laboratory there has furnished a set of *pararosanilin* samples which proved to be exceedingly good.

With this heterogeneous collection of samples it proved interesting to learn what the H-ion concentration was and whether there were any variations detectable which could be correlated with other significant data. It was expected to find the hydrogen-ion concentration quite high, but it was a matter of some surprise to discover how little variation there is in the reaction of the stain solutions. Table 1 is representative of the whole group. Determinations are by potentiometer, with a gold and quinhydrone electrode.

It is evident that when these solutions are fresh the H-ion concentration is quite uniform, in spite of the marked differences displayed in appearance and behavior. It is of interest also to note that after a period of time, due undoubtedly to inadequate sealing of bottles, there is a darkening of the solutions with a tendency to turn red or pink. That is accompanied by a small but marked rise of the pH, linked with a loss in the SO_2 , followed eventually by reabsorption of atmospheric oxygen.

The question of the optimum acidity in the solution is still debatable and calls for further investigation, which the writer hopes to undertake. So far the experience in this laboratory has been that the use of HCl in the dye solution is necessary with our American fuchsin. Lack of sufficient acidity causes foggy or smudged stains affecting the whole section, and the chromatin does not seem to differentiate and hold the stain as firmly as desirable.

Another point which should be discussed here is the matter of the sulfite. It was realized nearly two years ago that the reducing agent used was not actually NaHSO_3 (acid sodium sulfite) but rather the metabisulfite $\text{Na}_2\text{S}_2\text{O}_5$. The acid sulfite is extremely difficult to keep anhydrous and it is only available when specially prepared. The metabisulfite is the most closely related salt available commercially, altho still a product subject to a certain degree of decomposition. Potassium metabisulfite ($\text{K}_2\text{S}_2\text{O}_5$) is still more stable and uniform in composition, and was thought to be closely enough related to the sodium salts to warrant investigation. It has now been in use for more than a year in comparison with the homologous sodium salt in amounts which are comparable, 0.6 g. of $\text{K}_2\text{S}_2\text{O}_5$ to 0.5 g. of $\text{Na}_2\text{S}_2\text{O}_5$ for 100 cc. of the dye solution. The substitution of a different cation in the salt does not seem to impair the performance of the stain in any detectable way and gives us a much better tool for a closer control of the reaction. At the present time 0.5 g. of $\text{K}_2\text{S}_2\text{O}_5$ is used to 100 cc. of the dye solution, an amount which has

proved sufficient for a complete decolorization in 12 hours. It may be added that recent correspondence from England tells of some investigations carried out there on very much the same lines and with comparable results.¹

In the preparation of the solution it will be noticed that, as given in Margolena's schedule, the fuchsin is to be brought to a boil. This and other schemes have been tried and it is the writer's conviction that, in order to get as much as possible of the dye in solution, a better technic is to bring the water to the boiling point and to pour it onto the dye in a large-mouthed receptacle, to shake it up for a little while, to cool, and to proceed with the filtration. The second step of cooling to 25° C. is of no significance for quantities of approximately 100 cc.

Staining: As to a pretreatment we find that with the fuchsins available now there is no need for a special treatment of the section with dimethylcyclohexandione. The dyes are sufficiently selective and the reducing agent is powerful enough to eliminate whatever traces of the color might have clung to the cytoplasm.

While it has been suggested by others that 0.5 N HCl be used thruout, there is evidence that a *normal* solution as originally prescribed is more reliable. Some of the fuchsins in the present series do not respond as readily and neatly when the acidity in which the hydrolization and washing are carried out is unduly lowered.

The matter of the timing of the various steps deserves special consideration. An attempt has been made to establish quite definite limits for each one of them. It was found that plant materials generally require more time than animal tissues. This is particularly true with respect to the hydrolization process and the staining proper. A complete schedule as used in our laboratory, with all working details, is given below. The use of carefully prepared distilled water thruout is especially important. When a good Feulgen stained section comes out of the staining solution, it must show a definite faint purplish tinge. This is maintained thru the three sulfite washings and the color on the chromatin may finally be enhanced by a short soaking (10-20 minutes) in plain distilled water. Sections carried so far can be kept overnight in the water without noticeable damage.

Counterstaining: It has been suggested in the past that most of the common counterstains are satisfactory in the case of a Feulgen stain. Erythrosin, light green, and orange G have all been suggested. There are three main vehicles for a counterstain: water, alcohol, or

¹An account of this work is given in Revector Bull. of Stain Technique (published by Vector Manufacturing Co., London), 2, No. 3, 1936

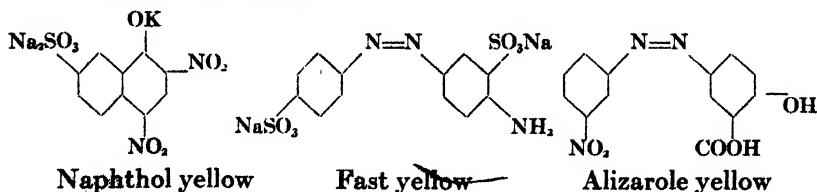
oil (usually clove oil). In this work the matter has been studied with the idea of meeting two major requirements: first, to obtain a counterstain which would permit maximum contrast; second, to secure a dye which would be readily soluble in water and as little as possible in alcohol.

To meet the first point, only green and yellow dyes were considered, as these colors are complementary to purple and magenta. Of the green dyes, light green was soon replaced with fast green, which is only half as soluble in alcohol and is much more resistant to fading. It can be used in 1% aqueous solution or 0.5% in 95% alcohol. In the alcoholic solution it is quite satisfactory for plant tissues, applied directly for two minutes after the last water step. In this case it must be remembered that water would remove it instantly; for this reason the extra dye must be washed out while completing the dehydration with 95% and absolute alcohol. Of the yellow dyes, orange G in 1% aqueous solution for five minutes proves very satisfactory on animal tissues, while it is not taken well by plant

TABLE 2. COUNTERSTAINS INVESTIGATED

Name	Dye Group	C.I. No.	% Solubility		Staining
			Water	Alc.	
1. Martius yellow	Nitro	9	4.57	0.16	Tends to replace nuclear stain.
2. Naphthol yellow	Nitro	10	8.96	0.025	Nuclear stain noticeably covered.
3. Fast yellow . . .	Azo	16	18.40	0.24	Too dark, smudgy.
4. Alizarol yellow	Azo	36	25.84	0.04	Tends to replace nuclear stain.
5. Orange II. . .	Azo	151	11.37	0.15	Too diffused.

material. As in the case of fast green, water will promptly remove the counterstain; therefore, it must be washed and dehydrated with 95% and absolute alcohol. Less satisfactory results were obtained with 1% aqueous solutions of five other dyes as indicated in Table 2. Of these five dyes the structural formulae of martius yellow and orange II are given in Biological Stains (Conn, et al., 1936). Those of the other three are as follows:



All these are acid dyes. Alizarole yellow was obtained in the form of its color acid (as shown above) which is not soluble in water, and had to be converted into its Na salt in order to employ it. These

dyes seem to deserve attention and it is hoped to have an opportunity in the near future of devoting more time to them.

It is mentioned above that dyes of high solubility in water should be selected. It is clear that under such conditions one can apply the counterstain after the last water rinse without further complication and with no fear of damage by the subsequent dehydration. This plan has advantages over the much used clove oil dye solutions. In this latter case one must dehydrate completely before applying the counterstain. The counterstaining must then be followed by a wash in xylene which, as in the case of the orange G, causes all the dye to precipitate immediately. This kind of trouble is particularly undesirable in cytological work.

A preliminary note concerning these recommendations has already appeared (de Tomasi, 1936). The detailed steps of the procedure follow.

PROCEDURE RECOMMENDED

1. *Preparation of Fuchsin Solution:*

Dissolve 0.5 g. fuchsin by pouring over it 100 ml. boiling distilled water. Shake thoroly. Cool to 50° C. Filter; add 10 ml. *N* HCl to filtrate. Add 0.5 g. $K_2S_2O_8$. Shake, close tight, store in the dark for 12–18 hours.

2. *Staining:*

Run down to water. Rinse in cold *N* HCl. Hydrolize in *N* HCl at 60° C., 4–5 min. Rinse in cold *N* HCl. Rinse in dist. water. Stain in the decolorized fuchsin (animal tissue 2 hr.; plant tissue 3–5 hr.). Drain and pass quickly to the first of 3 closed Coplin jars containing following acid solution: *N* HCl, 5 ml.; 10% $K_2S_2O_8$, 5 ml.; dist. water, 100 ml. Wash 10 min. in each. Rinse in dist. water. Counterstain; animal tissue 3–5 min. with 1% aq. orange G; plant tissue 1 min. with 0.5% fast green in 95% alcohol. Wipe off excess dye. Wash clean with 95% and dehydrate with absolute alcohol. Clear and mount in dammar.

It is hoped that this revised procedure for the Feulgen stain, together with the new fuchsin to be used in it, will prove a sufficient advance toward perfection of the technic to be of some definite value in histological and cytological work.]

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MICROSCOPIC SLIDES OF CAT TESTIS

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Thin (3 mm.) slices of cat testis were fixed for 48 hours in freshly prepared Karpechenko's fluid. This fixative was designed for use in plant cytology and was prepared by mixing 50 ml. of 1% aqueous solution of chromic acid, 4.5 ml. of distilled water, 5.0 ml. of glacial acetic acid, and 40 ml. of formalin¹.

Following fixation the material was washed for 24 hours in running water. Dehydration in ethyl alcohol, clearing in toluene, and infiltration with paraffin were carried out gradually. Paraffin (M.P.



Fig. 1. Metaphase of spermatogonial mitosis. 1250 \times .

Fig. 2. Spermatid showing deeply stained acrosome, caudal sheath, and derivatives of the centrioles. The derivative of the proximal centriole extends to one side of the caudal sheath. 1250 \times

Fig. 3. Later stage of spermiogenesis showing caudal sheath somewhat separated from the spermatid nucleus and in contact with the derivative of the proximal centriole at both sides. 1250 \times .

51° C.) sections were cut at 5 μ and stretched in water at 40° C. on slides coated with Mayer's albumin fixative. After the sections were freed from paraffin they were protected by a thin film of parlodion thruout the remainder of the process. The material was stained in Heidenhain's iron hematoxylin, being mordanted in iron alum for three hours and stained in 0.5% hematoxylin for an equal length of time. Destaining was effected by a 1% aqueous solution of iron alum. The procedure outlined in this paragraph is given in the reference noted below and differs only in the preparation of the staining solution.

¹Galigher, A. E. 1934. *Essentials of Practical Microtechnique in Animal Biology*. A. E. Galigher, Inc.

The usual procedure for staining sections in iron hematoxylin was changed only in regard to details of pH control. The usual solution of hematoxylin² was prepared. This was saturated with quinhydrone for direct determination of its pH in a quinhydrone electrode. The pH of this solution was then brought within the range from 7.00 to 7.06 by addition of saturated aqueous solution of lithium carbonate and of acetic acid. The regulation of the pH was effected immediately before using the solution. Excellent results were obtained by this method as early as one week after preparation of the hematoxylin solution—3 days after complete solution of the stain.

Fine preparations of mitotic figures were obtained (Fig. 1). The transformation stages of spermiogenesis were exceptionally clear (Figs. 2 and 3). The acrosome retained the stain while the material was destained sufficiently to bring out the finer details in the region of the neck (Fig. 2).

Samples of this material were fixed in Allen's "B-15", 10% formalin in physiological salt, and in Flemming's strong solution with acetic acid. These were treated exactly as and simultaneously with the Karpechenko fixed material, except that longer periods were allowed for mordanting and staining the Flemming fixed tissues. The highest degree of contrast in those stages of spermiogenesis which follow the attachment of the acrosome to the spermatid nucleus was shown in material fixed in Karpechenko's fluid.

The photomicrographs are of sections of cat testis fixed in Karpechenko's fluid and stained as described above. The hematoxylin solution had not acquired the color of port wine before regulation of its pH.

²Hematoxylin (C. P.) Coleman and Bell, Certification No. FH-11.

MOTOR END-PLATES

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ABSTRACT.—In the preparation of motor end-plates for class room work the use of sodium thiosulfate is suggested as a precaution against overstaining and as a regressive measure. Digestion of the connective tissue eliminates the tedious teasing and the possible destruction of the delicate nerve fibers. Mounting in a solution of gum arabic makes the slides more durable than glycerin jelly for class use. Whole neuromuscular spindles may be obtained by this method.

Preparation of motor end-plates requires considerable time and skill. Experience has proven that the usual gold-chloride-glycerin-jelly mounts for class room use are too fragile to last more than a short time and as a consequence the preparation of these slides must be made a part of the yearly routine. In an effort to eliminate the reduplication of technical procedure, a satisfactory method of preparation of motor end-plates has been used by the author in this laboratory which does not require the use of glycerin jelly as the mounting medium.

Intercostal muscles of a rabbit are obtained immediately after killing. Pieces should be cut small to insure rapid diffusion of the solutions. The staining method is a modification of the Ranvier technic in which small pieces of muscle are placed in fresh lemon juice for 30 minutes. They are rinsed quickly in distilled water, and placed in a bath of 1% gold chloride for 30 minutes. The muscle is washed in distilled water rapidly, and transferred to 10% formic acid for reducing the gold. The reducing must be done in the dark, and will require about six to ten hours. The exact timing must be partly determined by test dissections in glycerin and observation under the microscope. When the endings are well stained, and the muscle is red (not purple), the tissue is washed well in running water, and placed in a 5% solution of sodium thiosulfate for 15 or 20 minutes. Prolonged fixing in this solution tends to destain and, as a consequence, it must be done with care. It may be used as a regressive stage if the tissue is slightly over-stained. The tissue is washed in running water for an hour, and then placed in artificial gastric juice (powdered pepsin, 1 part; water, 1,000 parts; HCl, 3 parts). Digestion takes place better at about body temperature, and the tissue

will be soft in one or two hours. Judgment must be exercised from the texture of the muscle mass. It should be easily spread out with slight pressure under a cover glass. The gastric juice is then washed out by several changes of water. Running water should not be used, since the tissue is extremely flaccid and much of it may be washed away. It is then transferred to pure glycerin, which should be renewed three times in a period of about two hours. Very small pieces of the muscle are cut with scissors and placed on a clean absorbent cloth to drain. Each piece is transferred to a slide and covered with one or two drops of a solution of powdered acacia in water (about the consistency of glycerin) to which a few drops of formalin have been added. By gently pressing a cover glass down on the tissue it will flatten and bring the nerve fibers into view. Observation under the microscope will determine which of the slides have motor end-plates. After a period of time the cover glasses become well fixed, and the slides will stand ordinary handling of balsam and cedar oil mounts.

The slides may be sealed with gold size or thin balsam after they are thoroly dry. This prevents any damage which might be caused by the slides becoming wet. Altho some authors suggest the use of alcohol as a dehydrant, which makes it possible to mount the muscle tissue in balsam, this method was not found to be successful by the author. The lemon juice tends to swell the muscle cells and make them transparent. All available dehydrants caused a shrinkage, which resulted in the cells becoming opaque.

It is advisable to use sodium thiosulfate to prevent the tissue from becoming too dark. The reaction is probably: $2 \text{AuCl}_3 + 8 \text{Na}_2\text{S}_2\text{O}_3 \longrightarrow \text{Au}_2\text{S}_2\text{O}_3 \cdot 3\text{Na}_2\text{S}_2\text{O}_3 + 6 \text{NaCl} + 2 \text{Na}_2\text{S}_4\text{O}_6$, in which the gold complex is soluble. The formic acid must be well washed out, however, or there will be a white precipitate of sulfur formed within the tissue. The artificial gastric juice has already been mentioned as a decided advantage over the teasing method and makes it possible to turn out many more slides in a limited time. In the course of developing this technic, entire neuromuscular spindles were isolated. If done on a large scale, it would be possible to obtain them in large numbers.

A STABLE, HIGH-CONTRAST MORDANT FOR HEMATOXYLIN STAINING

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ABSTRACT.—Small quantities of sulfuric acid will stabilize iron mordants, used in hematoxylin staining, by preserving these solutions against oxidation. The presence of acetic acid in the mordant improves the specificity of the stain. A stable, high-contrast mordant is obtained when both acids are combined with ferric-ammonium sulfate. This mordant, used in combination with fresh alkaline solutions of hematoxylin, has been found especially effective in the staining of certain nuclear and cytoplasmic components of plant cells.

In the staining of chondriosomes, plastid primordia, chromonemata, and other structural elements of plant protoplasm with iron alum hematoxylin, an effort is usually made to obtain a maximum high contrast of the stainable structures. While the hematoxylin is generally used in combination with a 4% solution of ferric-ammonium sulfate, a 5% ferric chloride mordant may give equally good contrast. The following modification of the usual mordant has proved to be especially effective for discriminative hematoxylin staining:

4% ferric-ammonium sulfate (C.P.).....	500 cc.
Acetic acid (C. P.).....	5 cc.
Sulfuric acid (C. P., Sp. Gr. 1.84).....	0.6 cc.

Altho good results may be obtained by leaving slides in this solution for 2 hours, a 12-hour treatment is recommended.

Destaining is effected with a similar solution, diluted one-half for use:

2% ferric-ammonium sulfate.....	500 cc.
Acetic acid.....	5 cc.
Sulfuric acid.....	0.3 cc.

The acetic acid is added to mordant the stainable structures more vigorously and to obtain a greater specificity of the stain. Both cytoplasmic and nuclear components will be stained more precisely and in greater contrast with the more faintly stained background of hyaloplasm and karyolymph. The hematoxylin precipitates heavily in the chromatic material and the finished preparation shows a maximum contrast of black structure against a background which is white, gray, or tan according to the fixative used.

The sulfuric acid is added to prevent the oxidation of ferric sulfate. In theory only a slight excess of SO_4 -ions will insure a clear solution against precipitates, but in practice the quantity indicated above seems desirable. Such solutions have remained free of precipitates for a year on the shelf and should remain clear indefinitely. No injurious effects have been noted with this type of mordant even in the most delicate structures, in fact equally good results have been obtained when the concentration of this acid is increased to 1%.

Only distilled water is used in preparing the mordants and they are kept in clean, well-stoppered bottles. Aqua regia will quickly remove the iron oxide from containers in which the usual type of mordant has been stored.

The length of time in the stain is modified by too many variables to make a definite schedule possible. In general, the following conditions seem to apply:

With very fresh solutions of 0.5% hematoxylin, the staining time is about 4 to 8 hours. The freshest stains give the highest contrast, the stain becoming less specific with increasing age. Artificially "aged" hematoxylin solutions appear to be undesirable where the very maximum contrast is wanted. In all instances of hematoxylin staining it seems best to leave the slides in the stain for only the shortest effective time. As the hematoxylin solution becomes older, the staining time becomes correspondingly reduced. Noticeably better results are obtained with alkaline hematoxylin¹ than with more acid solutions.

When less contrast and purple tones are desired, the acetic acid is omitted from both mordants. In this case, however, following certain fixatives such as formalin-formic acid and F. A. A., black staining is obtained.

Acetic-mordants are not recommended for general histological work. For such purposes and for general low-contrast staining, where violet and silver-blue tones are especially effective, it seems desirable to use a 4% mordant (without acetic acid) for 2 hours, to stain as usual, and to differentiate with picric acid in 70% ethyl alcohol². Unused stain solutions which are 1 to 3 years old are often quite good for low-contrast staining with a silver-blue effect.

Hutner³ has recommended the use of H_2O_2 as a destaining agent for hematoxylin, this method having been found valuable in work on

¹Galigher, A. E. 1934. *The Essentials of Practical Microtechnic in Animal Biology*. A. E. Galigher, Inc., Berkeley, Calif.

²Tuan, H. C. 1930. Picric acid as a destaining agent for iron alum hematoxylin. *Stain Techn.*, 5, 135-8.

³Hutner, S. H. 1934. Destaining agents for iron alum hematoxylin. *Stain Techn.*, 9, 57-9.

protozoa. One can obtain a very low contrast of the hematoxylin on plant materials by this procedure or by using FeCl_2 . The H_2O_2 can be greatly activated as an oxidant by making it alkaline, but since the peroxide is very unstable in alkaline solution it must be handled cautiously (3% solutions as purchased have a pH of about 2.90 and 30% solutions may have an acidity greater than pH 2.30). The author has not found either acid or alkaline H_2O_2 destaining of noteworthy value with plant material, but FeCl_2 has been valuable in destaining nucleoli, especially following certain chondriosomal fixatives.

With Delafield's hematoxylin, 70% alcoholic picric acid seems to be decidedly preferable to the usual aqueous picric acid or HCl solutions for differentiation purposes.

PINACYANOL AS A SUPRA-VITAL MITOCHONDRIAL STAIN FOR BLOOD

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ABSTRACT.—Pinacyanol¹ is an excellent, almost permanent and very selective stain for mitochondria in supra-vital blood preparations. It may be used alone or in combination with neutral red for the study of fresh blood, bone marrow, spleen or lymph node smears. One drop of a 0.1% solution of pinacyanol in absolute alcohol, per cubic centimeter of the same solvent, is a satisfactory dilution for making stain films for blood with an average concentration of cells.

In 1933 Proescher² described briefly the nature, formula and staining qualities of pinacyanol when used as a histological stain for frozen sections of native or fixed tissues. No mention was made of its use as a supra-vital stain.

The writer, while studying some leukaemic blood by the supra-vital method, used pinacyanol among other dyes for the preparation of stain films and found that the mitochondria of the blood cells were selectively stained very deep purple or dark blue. At the same time it was noticed that these structures retained the dye for very long periods of time and that there was no fleeting or reduction of the dye so annoyingly characteristic of Janus green.

The films of stain were made in the usual manner by flooding clean, flamed slides with an absolute alcoholic solution of pinacyanol and draining off the excess. One drop of stock solution (0.1% dye in absolute alcohol) was used for every cubic centimeter of the same solvent. This dilution proved to be satisfactory for studying mitochondria in films of peripheral blood. Where many cells occur in smears such as in leukaemic blood or in spreads made from material obtained from spleen, bone marrow or lymph node punctures, it is necessary to increase the dye concentration. The strength of pinacyanol to procure an optimum staining of mitochondria must, for obvious reasons, be determined by each experimenter in a series of trial stains. Pinacyanol may be combined with neutral red for double stains and apparently does not inhibit the effect of the neutral red as does Janus green. The stain films should be made shortly

¹Obtained from the Eastman Kodak Company, Rochester, New York.

²Proescher, F. 1933. Pinacyanol as a histological stain. *Proc. Soc. Exper. Biol. and Med.*, **31**, 79-81.

before use since they do not seem to keep well altho the stock alcoholic solution, if kept well stoppered, does not deteriorate.

In a blood smear made upon a dye film of satisfactory concentration and sealed with salvoline, the mitochondria at first may be seen in some of the cells as untinted refractive rods or coccoid bodies which slowly assume a pale bluish color that increases steadily until it is intensely purple or blue. Mitochondria so stained have been observed unchanged in character two to four days later. In properly stained supra-vital smears, pinacyanol seems to be specific for mitochondria, since vacuoles, fat droplets and specific granules have not been observed to stain in the living cells.

It is true that the dye, like Janus green, is toxic and when too concentrated rapidly stains the nuclei and kills the cells. Pinacyanol tends to stain the nuclei of cells a lavender color even in optimum dilutions for demonstration of mitochondria. The nuclear tinting, however, is not necessarily a sign that death of the cell is imminent; since, as far as can be observed by comparison with unstained cells, the motility and behavior of the cell with a stained nucleus is unchanged for a surprising length of time. The polymorphonuclear neutrophiles appear to be affected first, especially those with the more highly segmented nuclei.

Likewise the nuclei of the other cells of the blood will become tinted after a time, but since the effect is so gradually produced, there is ample time for study of the cells. Even tho the nuclei may stain, the dye does not seem to alter their character as may be seen by comparing them with those of cells from unstained smears. In fact the nuclear staining is of considerable advantage since it offers a means of determining the relative fineness or coarseness of the chromatin masses in different nuclei. Such differences, when considered with other features of a given cell, aid materially in its identification. Since the dye is insoluble in protective saline solutions, such as those of Tyrode and Ringer, a satisfactory medium has not been found for perfusion of an animal with the dye for the staining of mitochondria in tissues before removal. Material from haemopoietic tissue, such as bone marrow which is ordinarily too thick to spread, may be thinned by a drop of serum or peritoneal fluid in which the dye is reasonably soluble.

A COLLOIDAL SILVER METHOD FOR NERVE CELLS AND PROCESSES, NEUROGLIA AND MICROGLIA

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ABSTRACT.—A method is described whereby nerve cells and processes, neuroglia and microglia may be stained using colloidal silver solutions (argyrol, silvol, 10% and 20%).

Fresh, unfixed brain tissue is stained in bulk in argyrol or silvol, and then dehydrated, embedded in low viscosity nitrocellulose, and sectioned. Before reduction the sections are treated with gold chloride to replace the silver. Sections are reduced in a formalin hydroquinone solution, fixed in sodium thiosulfate, dehydrated, and mounted in euparal. A method is described for removing the nitrocellulose before mounting.

No variation in the method was found to be necessary for the various species tested (rat, guinea pig, rabbit, and dog).

The routine silver methods used for the study of the nervous tissues are for the most part modifications of the original Golgi and Cajal reduced silver and silver pyridine methods, and of the Bielschowsky ammoniacal silver methods. These methods and their modifications involve the impregnation of the nervous tissue with some inorganic silver salt or complex, the reduction and deposition of the silver in the nervous elements, and the subsequent replacement of the silver with gold.

In the method herein described two new principles are introduced, first, the use of colloidal silver for impregnation and second, the replacement of the silver with gold before reduction.

Taft and Ludlum (1930) have shown that fresh brain tissue can be stained by means of a silver proteinate (argyrol). Dieterle and Neumann (1930) claim that a colloidal effect can be obtained by reducing with dilute formalin sections stained with ammoniacal silver solutions. Recently Bodian (1936) has reported a method by which nerve processes and nerve endings can be stained with a silver albumose solution (protargol) to which metallic copper has been added. The one advantage of using colloidal silver for impregnation is that interfering precipitates are rare.

The silver is replaced with gold before reduction, first, because colloidal silver reduces slowly and with difficulty, second, because

PLATE I

Fig. 1. Cerebral cortex of rabbit. 10% argyrol. $\times 85$.

Fig. 2. Same as Fig. 1, showing large pyramidal cells. $\times 360$.

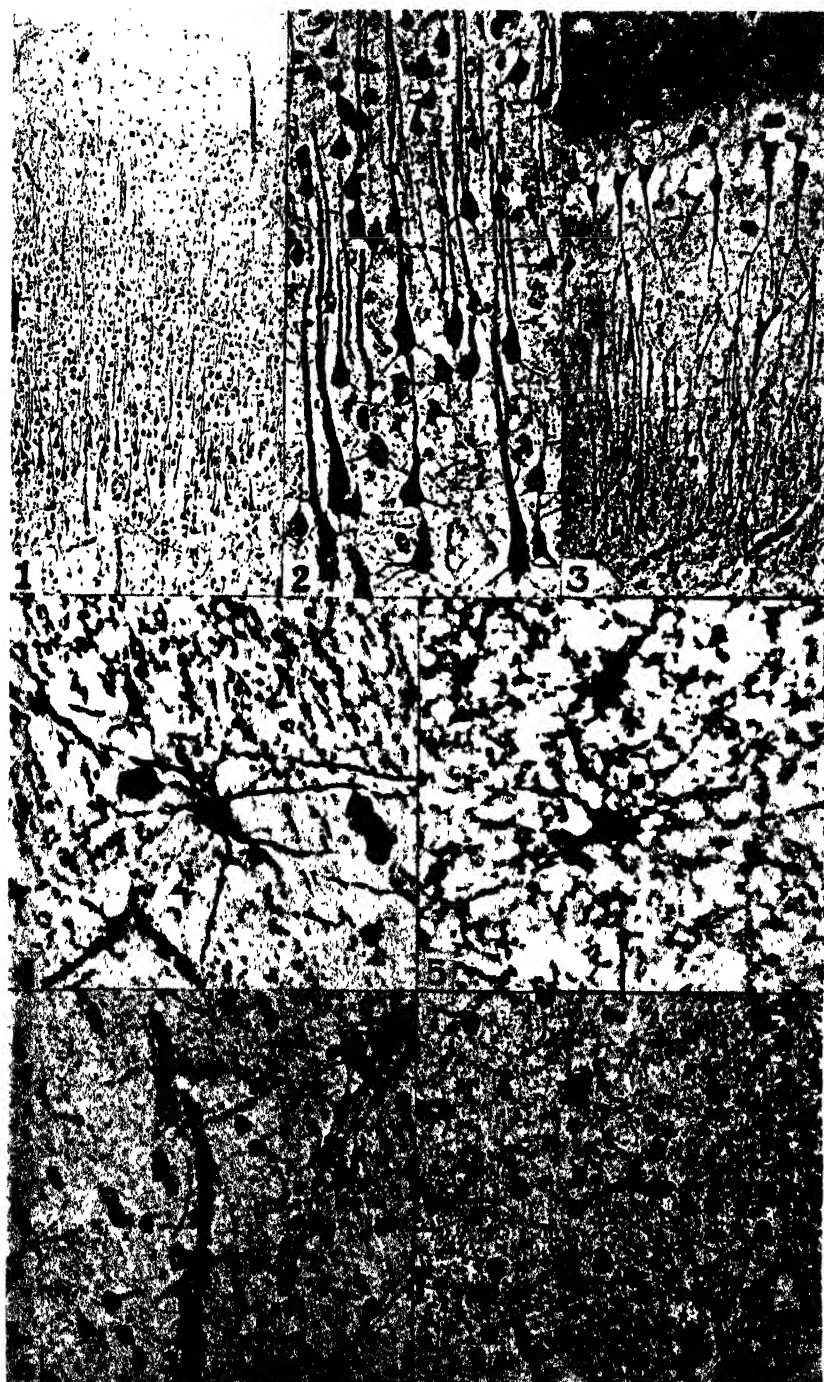
Fig. 3. Thalamus of rat, showing dividing processes of thalamic cells. 20% argyrol. $\times 200$.

Fig. 4. White matter of cerebrum of rat, showing a fibrous astrocyte. 10% argyrol. $\times 1000$.

Fig. 5. White matter of cerebrum of dog, showing a protoplasmic astrocyte. 20% silvol. $\times 1000$.

Fig. 6. White matter of cerebrum of rat, showing perivascular satellites (oligodendroglia). 20% argyrol. $\times 440$.

Fig. 7. White matter of cerebrum of guinea pig, showing microglia. 10% silvol. $\times 440$.



such treatment affords a method of destaining tissue which has been overimpregnated, and in addition gives a lighter background, which makes for better contrast.

The method stains nerve cells and processes a dark rose color on a grayish white background; cortical and thalamic cells and their processes show up particularly well. It may also be used for the demonstration of neuroglia and microglia, which it stains dark rose or black; protoplasmic and fibrous astrocytes, perivascular satellites, and interfascicular oligodendroglia are all shown.

METHOD

1. *Staining:*

Stain fresh brain tissue by placing thin slices (not over 3 mm. thick) in a freshly prepared colloidal silver solution (argyrol, silvol): it is necessary to have a large volume of solution: roughly 20 cc. for each sq. cm. of surface to be stained. The period of staining varies with the size of the pieces, the time since death, and the species of animal from which they were obtained; it varies from 48 hours to several weeks, and cannot be predicted. Overstaining, however, is of little consequence.

The tissue is stained when a freshly cut surface exhibits a dark brown color; and if a small piece is removed and crushed under a cover slip, nerve cells and processes will be found to be stained yellow.

2. *Dehydrating, embedding, sectioning:*

When the staining process is complete, remove the tissue and rinse quickly in 70% alcohol (2-3 min.), and then dehydrate, starting with 80% alcohol. (The colloidal silver diffuses out in the lower alcohols.) During dehydration allow three hours or over-night for each 5% step. From absolute alcohol place tissue in ether alcohol (ether and absolute alcohol, equal parts) for three to five hours, and then for one day each in 10%, 25%, and 50% low viscosity nitrocellulose.¹ Mount the pieces from 50% nitrocellulose on fiber blocks and harden in chloroform three to six hours. Leave in 85% alcohol (two changes) overnight and cut sections 10-20 μ , receiving them in 85% alcohol. (The colloidal silver may diffuse out in the lower alcohols; the higher alcohols cause undesirable softening of the nitrocellulose.)

¹R.S. $\frac{1}{2}$ sec. low viscosity nitrocellulose, viscosity 3/20-4/20, manufactured by the Hercules Powder Company, Parlin, New Jersey.

In making up the solutions in this laboratory allowance is made for the alcohol content of the nitrocellulose:

10%	10 g.	nitrocellulose,	50 cc. ether,	50 cc. absolute alcohol
25%	25 "	"	55 "	" 45 " "
50%	50 "	"	60 "	" 40 " "

3. *Treatment of sections with gold chloride, and reduction:*

Remove a section from 85% alcohol and float it on the surface of 0.5% aqueous gold chloride solution; the color of the section will change from yellowish brown to grayish white within one minute. As soon as this color change is complete, quickly remove the section and wash it rapidly in distilled water for 15 seconds, moving it back and forth by means of a glass hook. If, after final treatment a trial section is found to be too dark, wash succeeding sections longer than 15 seconds. For heavily stained sections the washing time may be 2 to 3 minutes, rarely longer.

After washing, place section immediately in a reducing solution of formalin and hydroquinone (hydroquinone, 0.3 g.; formaldehyde, neutral (Merck's) 30 cc.; distilled water, 70 cc.) for 2 to 4 minutes, rinse in distilled water 1 to 2 minutes, fix in 5% sodium thiosulfate 5 to 10 minutes, wash in distilled water 10 to 15 minutes, dehydrate in 95% alcohol 15 to 20 minutes, remove the nitrocellulose by means of ether alcohol, rinse off the ether alcohol with absolute alcohol, and mount in euparal.

To insure clarity and preservation of sections it is essential that the nitrocellulose be completely removed before the sections are mounted. This is most readily accomplished as follows:

a) Remove section from 95% alcohol by brushing it on to a piece of cigarette paper (non-ribbed variety) and smoothing out all wrinkles.

b) Apply the section to the slide, blotting it thru the cigarette paper with several thicknesses of filter paper.

c) By means of a brush apply absolute alcohol to the section thru the cigarette paper, brushing lightly back and forth. Blot with filter paper.

d) In the same manner apply several drops of ether alcohol.

e) Carefully peel off the cigarette paper (quickly, to avoid drying of section) and drop ether alcohol gently on the section until every trace of nitrocellulose is gone.

f) Rinse off the ether alcohol with absolute alcohol, wipe off the alcohol around the section, apply a drop of euparal and a cover slip.

By this method different tissue elements may be stained by varying the concentration of the colloidal silver solution. Thus, it has been found that:

(1) 10% argyrol stains nerve cells and processes and astrocytes.

(2) 20% argyrol stains nerve cells and processes, astrocytes, oligodendroglia and microglia.

(3) 10% silvol stains nerve cells and processes, astrocytes and microglia.

(4) 20% silvol stains nerve cells and processes, astrocytes and oligodendroglia.

4. *General precautions:*

a) The colloidal silver solutions should be freshly prepared before use. (Grind crystals to a powder and dissolve in distilled water.) Argyrol four or five months old stains very rapidly (probably because of the gradual liberation of free ionic silver), but is prone to produce disturbing precipitates.

b) Tissue should be kept in the dark during staining and dehydration.

c) Material may be kept in the colloidal silver several months without undue overstaining; but as soon as the tissue is removed from the silver solution the procedure as outlined above should be carried thru without interruption.

d) Sections should be manipulated only with glass hooks.

REFERENCES

- BODIAN, D. 1930. A new method for the demonstration of nerve processes and nerve endings in mounted paraffin sections. *Anat. Rec.*, **65**, 89.
DIETERLE, R. R. and NEUMANN, M. 1930. New demonstration of Hortega cells. *Arch. Neurol. and Psychiat.*, **24**, 1154.
TAFT, A. E. and LUDLUM, S. D. 1930. A method for staining unfixed brain tissue with silver. *Proc. Soc. Exp. Biol. and Med.*, **27**, 582.

SIMPLE AIDS TO MICROSCOPE ILLUMINATION

D. B. O. SAVILE, *Abbotsford, Quebec, Canada.*

A Mounting for the Reflecting Prism. The somewhat delicate pivots of the microscope mirror mounting make it unsuitable for carrying the greater and unbalanced weight of the reflecting prism. For ease of adjustment the prism should pivot about the center of the reflecting plane, which is far from the center of gravity. Tight pivots and large bearing surfaces are needed to overcome the tendency to slip.

A suitable clamp of the type illustrated in the accompanying drawing can be constructed easily from fairly heavy gauge sheet copper or brass. First cut a strip to form the saddle A, which holds the prism; after bending the strip into a U just wide enough to slip over the prism, solder small distance pieces (shaded in the elevation view) inside the arms of the U to hold the face of the prism about $\frac{1}{4}$ in. away from the base of the saddle; this prevents the reflecting surface from being scratched and facilitates attachment of the pivots in the required place. The corners of the saddle are bent over at B to keep the prism in place. A small strip C is soldered on as shown to lend rigidity; it should preferably be made with a slight curve in the center; pressure on this curve spreads apart the arms of the U, facilitating removal of the prism for cleaning.

Bend another strip, for the clamp D, to the shape shown in the plan view. Make cuts about $\frac{1}{8}$ in. deep into the lower side at the points E, E, turn up the metal as shown and run a little solder into the corners where the overlap occurs; this gives the necessary rigidity. The bearings, perhaps, present the chief difficulty. If a lathe is available, steel spigots G and brass or bronze collars H can quickly be turned up; otherwise one must make-shift with anything available. Personally, I used small brass terminal nuts from a dry cell and the tops of two broad-headed nails. A little work with a fine flat file resulted in a smooth fit. The finished spigots and collars are soldered in position. The soldering is best done without a soldering bit. Clean the surfaces to be joined, apply saturated zinc chloride, heat and coat with solder; finally apply more zinc chloride, superimpose the pieces and press together in the flame till the solder runs. In attaching the spigots to A the preliminary tinning should be done before the distance pieces are soldered on inside; when the actual joining is done, direct a pointed flame downward onto the spigot and

stop heating as soon as the solder flows; otherwise the distance pieces may become unsoldered.

The means of attachment to the microscope depends upon the make of instrument; a split plug of the type shown at J is commonly used. If the prism is always to be used with the same microscope,

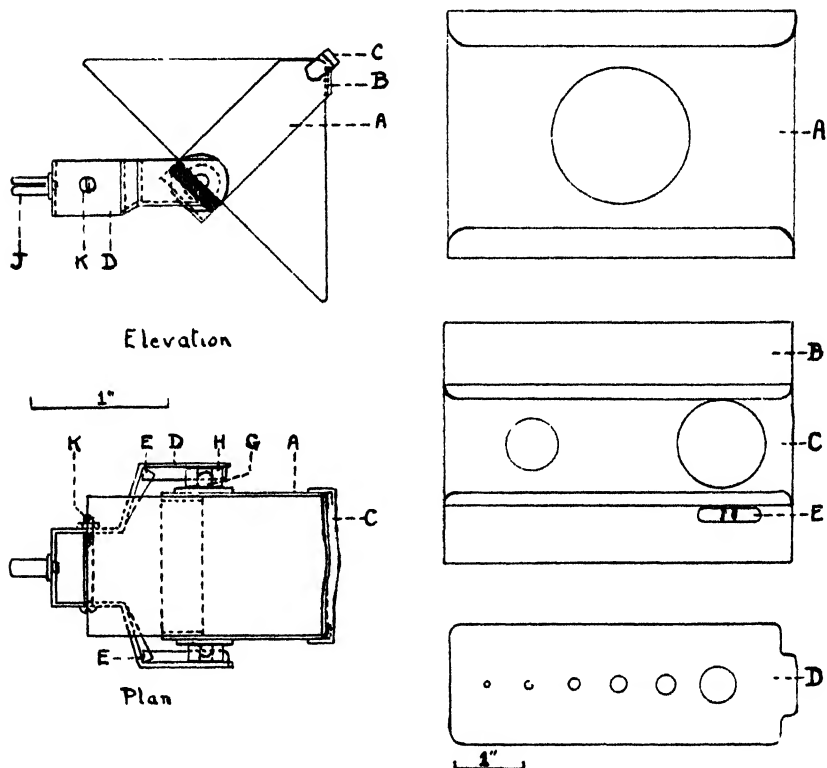


Fig. 1

Fig. 2

Fig. 1. A mounting for the reflecting prism.

Fig. 2. Diaphragms for the microscope lamp.

the attachment of the mirror mounting may be used. Alternatively a split plug may be fitted and pressed into a hole in a hardwood block, the clamp D being swung down into a vertical position and the block being shaped to fit between the feet of the microscope stand; the only disadvantage of this method is that the prism does not move with the microscope. A suitable plug may be found on a derelict microscope or may be made by cutting down a pin from an electric

plug of the old type which had split pins as connectors in place of the flat strips commonly used today.

To complete the mounting, the clamp is drilled to take a small bolt K; the nut on this is tightened until the prism moves fairly stiffly. Finally it should be noted that alterations in some of the dimensions will be necessitated by different sizes of prism.

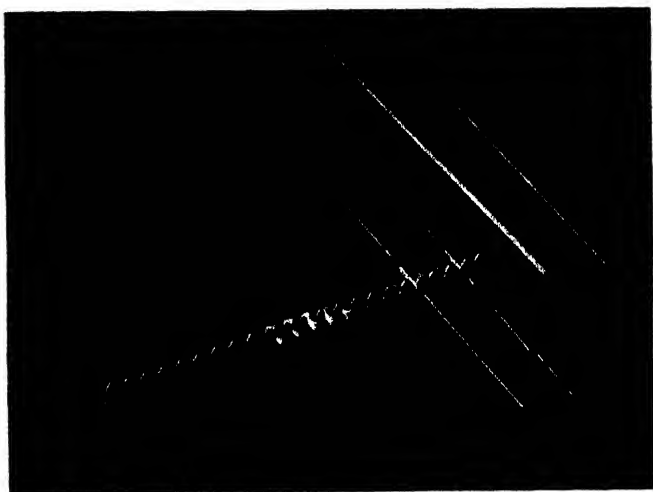
Diaphragms for the Microscope Lamp. It is convenient to have a reasonably compact set of lamp diaphragms which will allow a full range of stops without having to remove one slide and insert another or alter the relationship of lamp and condenser. The set here described has been found satisfactory and is simple to make. The necessary material was obtained from 9×12 cm. film pack cases, which are made from thin, easily-worked sheet metal. Part A (see Fig. 2) is made from the back part of the casing, the ends being trimmed and the sides being folded over to give a sliding fit over B, which is made from the piece which presses the films against the face of the pack. Part A is mounted on the lamp by whatever means prove most suitable for the type of lamp employed; it should, of course, be immediately in front of the ground glass. Holes are cut in B slightly larger than those in C; C is formed with the edges turned over to form guides for D and is then sweated onto B; its back and the corresponding front portion of B are rubbed bright with fine emery paper, the surfaces are painted with saturated zinc chloride, chips of solder are distributed over B, and C is placed on top. The two parts are placed in position on any convenient support, C is pressed firmly down and heat is applied from below with a bench burner until the solder flows freely; the pressure is maintained on C until the solder has set, when the whole is washed thoroly under the tap and dried. A small loop E is soldered on to B to form a handle. D is cut to slide in C and is turned up at the end for convenience of manipulation. The larger holes can be marked out by scribing circles with a pair of dividers and are easily cut out with a small pair of curved scissors. The smaller holes must be drilled; either sweat the piece D to a strip of scrap brass or clamp it between blocks of hardwood for the operation; the former is recommended as there is less likelihood of the holes being out of position. To avoid tearing such thin metal the sheet must be firmly held and the drill turned fast but fed very gently.

Before making any of the holes scribe center lines the full length of each part and make certain that these are superimposed in assembling; failure to get the various openings correctly aligned necessitates extra manipulation of the mirror.

The holes in D must be spaced so that when one is centered over the smaller of the two holes in C no light shows thru the adjacent ones. The sizes of openings required depend upon the objectives and condenser employed, but the range illustrated covers most requirements.

NOTES ON TECHNIC

A HANDY SLIDE AND COVER SLIP RACK.—The cartridges of 25 staples each, manufactured by the E. H. Hotchkiss Co. for their No. 1 Automatic Paper Fastener and available at most stationers', form a cheap and handy holder for clean slides and cover slips. The car



tridges may simply be laid on their backs on the table, or nailed to a small board. A shallow Stender dish, with a portion of a cartridge in the bottom, will serve as a Columbia staining jar for many purposes.—K. W. COOPER, Dept. of Zoology, Columbia University, New York, N. Y.

THE CELLOSOLVE-NITROCELLULOSE TECHNIC.—The use of ethylene-glycol-monoethyl ether ("Cellosolve", Carbide and Carbon Chemical Co.) as a matrix solvent may be recommended in cases of difficult penetration.

For use with large blocks of nervous tissue the following procedure has been found satisfactory:

1. Fix and wash as usual.

2. Place block in several changes of cellosolve.

3. Run up the block in graded concentrations of "nitrocellulose ½-sec." (Hercules Powder Co.) to a fairly heavy concentration of solute. (The specimen may be left for an indefinite period at this stage without danger of drying up since the boiling point of cellosolve is quite high. This quality also inhibits bubble formation in ventricles and other "blind spaces".)

4. Transfer piece to alcohol-ether solution of "nitrocellulose ½-sec." in about 10% concentration. Thicken gradually, block up and harden in chloroform.

Any of the usual stains may be used after this method. It has been our custom to follow dehydration in 95% alcohol by removal to terpineol if the cellulose matrix is to be preserved. The sections are placed on the slide and flushed with xylene and then mounted in balsam. If the matrix is to be removed the section is placed from 95% alcohol on the slide and flushed with absolute alcohol and then methyl salicylate which removes the matrix and clears simultaneously. FRED A. METTLER, CECILIA C. METTLER AND F. C. STRONG, University of Georgia School of Medicine, Augusta, Ga.

THE DIOXAN PARAFFIN TECHNIC.—The cutting of sections of 2 by 3 inches square in paraffin can be accomplished with reasonable care by the substitution of 1, 4 diethylene oxide ("Dioxan", Carbide and Carbon Chemical Company) for alcohol and xylene in the embedding process.

For the embedding of large pieces of nervous tissue (and presumably other materials rendered friable by xylene) the following procedure is recommended.

1. Fix and wash as usual.
2. Place specimen in dioxan changing occasionally until anhydrous copper sulfate is no longer turned blue by the discarded dioxan. (The tissue may be conveniently left in this fluid for weeks.)
3. Embed in paraffin dissolved in dioxan in the same way in which one uses xylene or chloroform.
4. Section and mount.
5. Dissolve paraffin from section with dioxan and transfer to water. Stain as usual and mount.

This method possesses the advantage of preserving fine detail and keeping large blocks of the tissue soft.—FRED A. METTLER, F. C. STRONG AND CECILIA C. METTLER, University of Georgia School of Medicine, Augusta, Ga.

ALUM COCHINEAL-HEMATOXYLIN STAIN.—In using Delafield's hematoxylin for staining *toto* mounts the dark blue color of internal structures obscures many details; or if the destaining process is carried far enough to avoid this many structures completely lose their stain. Excellent slides have been made of trematodes and nematodes *in toto*, and of sectioned flatworms by using three parts of alum cochineal and one part of Delafield's hematoxylin. The recommended procedures for several different objects are:

	Alum Coch.	Delaf. Hematox.	H ₂ O	Time
Sectioned material	3 pts.	1 pt.	10 pts.	20-40 min.
Trematodes (<i>toto</i>)	3 pts.	1 pt.	25 pts.	2-3 hrs.
Nematodes (<i>toto</i>)	3 pts.	1 pt.	50 pts.	12-24 hrs.

Tissues should be passed thru acid and alkaline alcohol as when using straight Delafield's. Since alum cochineal is not taken out as rapidly as the hematoxylin, one gets a beautiful contrast in colors—ranging from light pink to dark purple.

This stain should be used after fixatives containing acetic acid, such as Beauchamp's aceto-formo-alcohol. It usually deteriorates after a few days.—BRUCE D. REYNOLDS, University, Virginia.

THE USE OF ACETATES AS A MEANS OF REMOVING AIR BUBBLES FROM LACTO-PHENOL MOUNTS OF FUNGI.—While working on species of *Chaetomium* difficulty was frequently encountered when the material was mounted in glycerin jelly or lacto-phenol because air bubbles would be held by the setae and the spores. This difficulty was overcome by adding a drop of methyl or ethyl acetate to the material. The spores and setae were wetted almost instantly.

It was found convenient to put a drop of Amann's lacto-phenol (lactic acid, 20 cc.; phenol, 20 g.; glycerol, 40 cc., and water, 20 cc.) on the slide; a bit of the fungus containing several perithecia was then placed on the slide in the lacto-phenol. The drop of methyl or ethyl acetate was then added, wetting all the perithecia. They could then be dissected in the lacto-phenol as the acetate evaporated rapidly and the lacto-phenol took its place, wetting the perithecia and spores. After dissection, a piece of glycerin jelly was placed on the slide, heated, and the mixture of glycerin jelly and lacto-phenol covered with a cover glass without the annoying presence of bubbles.

The low viscosity of the acetates combined with their low surface tension should be of value where great masses of spores are encountered which will not become wetted, when air bubbles are held in the mycelium, or when spores, by their presence on the surface of a liquid, obscure other features.—R. H. TSCHUDY, University of Wyoming, Laramie, Wyoming.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the July number of this Journal.

STAINS CERTIFIED JUNE 1, 1936 TO AUG. 31, 1936*

Name of dye	Certification No. of batch	Dye Content	Object of tests made by commission†	Date approved
Congo red	NQ 6	90%	As histological counter-stain	June 30, 1936
Wright's stain	LWr 7	—	As blood stain	July 14, 1936
Methyl green	NG 11	80%	In histology and as constituent of Pappenheim stain	July 15, 1936
Bismarck brown	NN 6	55%	As histological and bacteriological stain	July 18, 1936
Crystal violet	NC 17	94%	As histological, cytological and bacteriological stain	July 22, 1936
Crystal violet	LC 11	97%	As histological, cytological and bacteriological stain	July 22, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

BOOK REVIEWS

GUYER, MICHAEL F. **Animal Micrology.**, 4th ed. 6 x 9 in., 331 pp. Cloth. 76 illustrations. Index. University of Chicago Press, Chicago, Ill. 1936. \$2.50.

This well-known book needs no introduction to workers in microscopic technic. The new edition is about 30 pages longer than the 3rd and has generally been brought up to date. Chapter VII of the new edition is entirely new, dealing with the dioxan technic, a procedure which has come into use in histology since the last edition appeared. Several minor changes with added material have been made thruout, especially in Chapter XVII on embryological methods, Chapter XVIII on cytological methods, and Appendix B which contains formulae of stains and various reagents —*H. J. Conn.*

MICROTECHNIC IN GENERAL

PETRUNKEVITCH, A. and PICKFORD, G. E. **On the relative acidity of histological fixing fluids.** *Anat. Record*, 65, No. 4, 461-6. 1936.

The hydrogen-ion concentration of 34 standard fixing fluids was tested with a glass electrode and in several cases colorimetrically. With the exception of aq. osmic acid and 'neutralized' formalin none of the fluids have a pH higher than 4.9, while the most acid fluid, namely Gilson's sublimate mixture, gave a pH of about 0.0. The pH of mixtures containing formalin varies with that of the formalin used. Acidity as such, has nothing to do with fixation of mitochondria in view of the fact that Champy's fluid is quite acid (pH 1.5) and Flemming's without acetic still more so (pH 1.2).—*Author's Abstract.*

DYES AND THEIR BIOLOGICAL USES

GIROND, A., LEBLOND, C. P., RATSIMANANGA, R. and RABINOWICZ, M. **L'acide ascorbique ou vitamine C dans la cellule et sa detection.** *Protoplasma*, 25, (1), 115-23. 1936.

Szent-Györgyi had shown that the suprarenal cortex macroscopically blackens with silver nitrate and attributed this reducing action to the presence of vitamin C. The authors have found that this reaction can be used histologically to detect vitamin C in the cells of various tissues. The method is not described in detail except that the silver nitrate is acidified and the specificity of the reaction is diminished by heat and light. The reaction results in the formation of blackened granules either about the Golgi bodies or distributed thru the cytoplasm. It occurs abundantly in the suprarenal cortex, corpus luteum, interstitial cells of the testes, and the glandular part of the hypophysis. When the guinea pig from which tissues are taken has been deprived of vitamin C in its diet, the histological picture gives indication of this deprivation. The silver nitrate reaction occurs only in the presence of active vitamin C.—*R. Chambers.*

GUILD, R. and RAPPORT, D. Calorigenic action of methylene blue during muscular exercise. *Proc. Exp. Biol. & Med.*, **34**, 459-61. 1936.

The intravenous injection of 10 mg. of methylene blue per kilo in a dog was followed by a 42 per cent increase in metabolism, predominantly at the expense of the oxidation of carbohydrate.—*M. S. Marshall*.

HANUT, C. and FAUTREZ, J. Études sur les variations de dispersion des colorants. *Protoplasma*, **23**, 93-108. 1935.

This study concerns the dispersibility of dyes in solution. Trypan blue in concentrations from 0.25 per cent to 2 per cent is relatively stable, an increase in concentration increases very slightly the dimensions of the particles but the solution does not tend to flocculate. The same is true of ammoniacal carmine, Ink R. A. L. (a specially prepared India ink), used by Girard and Cordier on nephric tubules. On the contrary, brilliant Congo R and particularly benzoazurine are completely peptized only in the more dilute solutions; above a critical point they tend to flocculate. Blood serum tends to increase the dispersion of the dyes mentioned in concentrations below 1 per cent; above that the particle size approaches that in aq. solutions. Other colloidal solutions, such as ovalbumin, diluted 100 times, dextrinea 0.1% and gum arabic 0.05 per cent definitely decrease the dispersion.—*R. Chambers*.

LISON, L. Une réaction micro et histochimique des esters sulfuriques complexes, la "réaction metachromatique". *Bull. Soc. Chim. Biol.*, **18**, 225-30. 1936.

Sulfuric acid esters of high molecular weight (e.g., chondroitin sulfuric acid of cartilage) change the color of very dilute solutions (1:10000 to 1:50000, solvent not given) of certain metachromatic dyes, among which are toluidine blue and brilliant cresyl blue. This "metachromatic color change" is an extremely sensitive and strictly specific test for these substances and their salts. It must be distinguished from the "ionic color change" caused by alkaline solutions. The author gives 2 ways of distinguishing these color changes. (1) Extraction by a hydrocarbon: shake the solution which has changed color with a little benzene or toluene; if the solution becomes colorless while the solvent becomes colored, it indicates an ionic color change, whereas if the solution remains colored, it is a case of metachromatic color change. (2) Action of heat: raising the temperature changes the equilibrium between the 2 forms of the dye in favor of the "normal" form at the expense of the metachromatic one and does not affect the equilibrium between the ionic forms. The former is reversible and the color reverts to the metachromatic form on cooling. The sensitivity of the "metachromatic reaction" is thus increased for weakly chromotropic substances by cooling the test solution before use. The sensitivity of the reaction diminishes with decreasing molecular weights of the esters.—*L. Farber*.

MILLS, M. A. and DRAGSTEDT, C. A. Bromsulphalein dye retention test as a measure of functional activity of reticulo-endothelial system. *Proc. Soc. Exp. Biol. & Med.*, **34**, 228-31. 1936.

Functional impairment obtained by blockading the reticulo-endothelial system was measured by injecting dogs with 2 mg. of bromsulphthalein per kilo of body weight intravenously and taking blood samples 5 and 30 min. later. The dye was detected in plasma by adding 2 drops of N/10 NaOH. If the reaction was obscured by reagents used for blocking precipitation, acetone was used before alkalizing. Dye retention appeared to give a direct measurement of the effectiveness or degree of blockading.—*M. S. Marshall*.

ANIMAL MICROTECHNIC

BROWN, A. F. and KRAJIAN, A. A. Histologic demonstration of uremia by precipitation of xanthidrol urea in tissues. *Arch. Path.*, **21**, 96-9. 1936.

An excess of urica in the tissues may be demonstrated by the production of xanthidrol urea crystals by the following technic: Cut thin (2-3 mm.) blocks of tissue. Immerse 5-6 hr. in fresh solution of xanthidrol (5 g. in 100 cc. glacial acetic acid). Wash in running water 1 hr. Fix in 10% formaldehyde 6 hr. (Rapid fixation by heat may be used.) Wash in tap water. Cut frozen sections

5-10 μ . Transfer to glass slide. Pour several drops of dehydrated alcohol over the section from dropping bottle and blot. Repeat process. Cover with thin pyroxylin (celloidin). Fix by blowing over slide. Stain in a 1% aq. solution of eosin for several minutes. Wash in tap water. Dehydrate 3 times with abs. alcohol. Place in carbol xylene for 2 min. Clear in 2 changes of pure xylene, 1 min. each. Mount in dammar.—*Lall G. Montgomery.*

CHANG, M. A formol-thionin method for the fixation and staining of nerve cells and fiber tracts. *Anat. Record*, 65, No. 4, 437-42. 1936.

The nervous tissue can be fixed and stained simultaneously in 0.1 or 0.5 g. of thionin (Grubler) dissolved in 100 cc. of 10% formaldehyde for a few days up to 2-3 months. Then it is rinsed with dist. water, dehydrated in graded alcohol and embedded either in paraffin or in celloidin according to the usual technic. In so doing, the fiber tracts are stained red and cell bodies blue. Such a differentiation turns out more brilliantly if both fixation and dehydration be much prolonged.—*Author's Abstract.*

LISON, L. Sur la recherche histochimique des oxydases par la réaction du bleu d'indophénol. Le cas des lipides. *Bull. Soc. Chim. Biol.*, 18, 185-9. 1936.

The blue coloration taken on by certain fatty substances on treatment with the Nadi reagent (α -naphthol + dimethylparaphenylene-diamine) is not due to a selective action by the indophenol blue in the reagent resulting from its oxidation by atmospheric O_2 . It results from an oxidation of the Nadi reagent by the fatty substances themselves, or to be more exact, by a substance contained in the fatty material. In this case, however, it is not a question of an oxidase but of an oxidizing substance, very probably an organic peroxide, formed by the oxidation of unsaturated lipids. This fact calls attention to the possibility that the Nadi reaction may not always specifically indicate the presence of an enzyme.—*L. Farber.*

NARAT, J. K. A new method for preparing multicolored corrosion specimens of the kidney. *Trans. of Chicago Path. Soc.*, Oct., 1935. *Arch. Path.*, 21, 120. 1936.

After the blood vessels and ureter of the kidney have been thoroly flushed with water, they are filled with stained solutions of a vinyl resin in acetone. The organ is immersed in water for 24 hrs., a procedure which solidifies the injected solutions. The kidney is placed in conc. commercial hydrochloric acid for 4 days. All the tissues are digested and only casts of the blood vessels, ureter and calices containing the injected substances remain. By employing acid-fast dyes, multi-colored specimens may be obtained.—*Lall G. Montgomery.*

SCHOUR, I. Measurements of bone growth by alizarin injections. *Proc. Soc. Exp. Biol. & Med.*, 34, 140-1. 1936.

The rate of apposition of bone can be followed by the use of injections of sodium sulphalazate. Rats 3 months old were given 0.5 cc. of 2% alizarin red S, C. I. No. 1034, (Coleman and Bell Co.) 4-6 days apart and were killed 2 days after the last injection. The dose ranged from 39-62 mg. per kg. Microscopic examination of ground transverse sections of the mid-root level of upper molar teeth showed a red line in the alveolar bone that was calcifying at the time of each injection.—*M. S. Marshall.*

MICROORGANISMS

BROOKE, J. W. Possible bipolar nuclear distribution in bacteria. *Proc. Soc. Exp. Biol. & Med.*, 34, 185-8. 1936.

Definite granules were demonstrated in a number of bacteria grown in the presence of sugars, altho some of the sugars were not utilized by the organisms tested. With a wax pencil, mark a circle on a slide prepared as for a flagella stain and add 2 loopfuls of sterile water followed by a loopful of culture. Air dry, and flood with a 10% alc. basic fuchsin. Keep moist with drops of 95% alcohol for 7-10 min. Wash with water, place a drop of acid alcohol at the periphery of the ring and immediately wash it off with a gentle stream of water. Air dry and

examine. With *Proteus vulgaris*, methylene blue, crystal violet, carbol fuchsin, 0.1% basic fuchsin and malachite green failed to give granule stains equal to those secured with 10% basic fuchsin.—*M. S. Marshall.*

BUTT, E. M., BONYNGE, C. W. and JOYCE, R. L. The demonstration of capsules about hemolytic streptococci with India ink or azo blue. *J. Inf. Dis.*, 58, 5-9. 1936.

While India ink has been used often in the past for outlining bacterial capsules and organisms, no consideration has been given to the action of diluents upon the size of the capsular zone. An improved India ink technic is given with azo blue as an alternate choice. An imported product, Peliken ink No. 541 (Günther Wagner) is used; Higgins waterproof ink can be used only after centrifuging. The hemolytic streptococci are grown on plates of veal infusion agar (pH 7.2) with 5% defibrinated sheep blood and 20% ascitic fluid added. Incubate 7-8 hr. at 35° C. Make a thin suspension of the organism in 1 drop of 6% dextrose on a clean slide, add 1 small drop of the ink, mix, and spread by using the edge of another slide (as in making a blood film). Dry and counterstain with 1:1 stock methylene blue solution (no further details specified) and methyl alcohol, or fix in methyl alcohol and counterstain with an aq. solution of any dye selected. Wash off the excess stain and allow to dry without blotting. Azo blue (Coleman and Bell) is used in the same manner in the form of a 1% solution in 6% dextrose. It gives larger capsular zone, but a coarser background.—*J. A. de Tomasi.*

CLAUBERG, K. W. Ueber einige ergänzende technische Einzelheiten der neuen Indikator-Tellurplatte zur makroskopischen Diphtherie-bazillen-diagnose. *Zentbl. Bakt., I Abt. Orig.*, 135, 529-30. 1936.

Some difficulty has been experienced recently in connection with the new "indicator-tellur" plating medium (Clausberg, *Zentbl. Bakt., I Abt. Orig.*, 134, 271. 1935) for the recognition of diphtheria organisms macroscopically. The trouble is with the indicator (water blue). Instead of a uniform marine (navy?) blue when the medium is acid, the color often turns dark reddish violet. After incubation, the plate becomes dark dirty green which makes differentiation of the diphtheria organism difficult. In cooperation with the firm Dr. G. Grubler & Co., Leipzig, a new C. P. preparation has recently been developed called "Standardized water blue 6B extra P" which is stronger than previous products so that it can be used in a stock concentration of 1.75% instead of 2%. A deep blue circle or halo is characteristic of a diphtheria colony.—*J. A. de Tomasi.*

FUKUMOTO, K. Studies on the vital staining of bacteria. I. Influence of pigment on the growth of tubercle bacilli and non-pathogenic acid-fast bacilli cultivated in Kirchner's medium to which Ajinomoto was added. *J. Orient. Med.*, 23, 34. 1935.

This is a report on 12 dyes investigated as to their bacteriostatic effects upon the tubercle organism and other acid-fast bacteria. Safranin and trypanflavin proved most toxic, while methylene blue, gentian violet, eosin, fuchsin and scarlet red were less so in the order named. (Cited from *Zentbl. Bakt., I Abt., Ref.*, 121, 310. 1936.)—*J. A. de Tomasi.*

FUKUMOTO, K. Studies on the vital staining of bacteria. II. Pigment affinity of tubercle bacilli and non-pathogenic acid-fast bacilli. *J. Orient. Med.*, 23, 35. 1935

Using for comparison tubercle bacilli and non-pathogenic acid-fast organisms, 20 dyes were tested for their bacteriostatic properties and stainability of the granules. It was found that most of the strains including the tubercle bacillus, could be stained vitally since growth continued long after adsorption of the dye had become evident. (Cited from *Zentbl. Bakt., I Abt., Ref.*, 121, 311. 1936.)—*J. A. de Tomasi.*

GUITTONNEAU, G. and BRIGANDO, JEANNE. Sur une résistance aux colorations de certains corps microbiens acquise par chauffage dans le lait. *Compt. Rend. Acad. Sci.*, 202, No. 19, 1622-4. 1936.

Most of the bacteria in a sample of milk which has been submitted to considerable heat (1 hr. at 100° C. or 20 min. at 120° C.) will not stain but appear as white spots against the colored background. This can be shown with 1% aq. methylene blue, as well as with gentian violet, Unna's blue, erythrosin, or hematoxylin. It is thought that this particular behavior is dependent upon certain unknown conditions of the cells, and upon the development of definite sensitizing factors brought about by thermic alteration of the phosphocasein complexes in raw milk.—*J. A. de Tomasi.*

JOHNSON, C. M. A rapid technique for iron-hematoxylin staining requiring no microscopical control of decolorization. *Amer. J. Trop. Med.*, 15, 551-3. 1935.

This new technic, suggested as a rapid stain for amoebae, includes the following steps: Fix 10 min. in sat. Schaudin solution, plus 5-10% acetic acid; treat 5 min. in iodized alcohol (95% alcohol plus I, until of a port wine color); wash in 70% alcohol, 5 min.; rinse in water, 1-3 min.; soak in 4% iron alum, 15 min.; rinse in water, 1-2 min.; stain with 0.5% aq. iron hematoxylin, 10 min.; decolorize with 0.25% iron alum; wash in running water and dry.—*J. A. de Tomasi.*

KEH-HUNG, LI. Antigenicity of staphylococcal toxin detoxified by the photodynamic action of methylene blue. *Proc. Soc. Exp. Biol. and Med.*, 34, 659-61. 1936.

Grubler's methylene blue was used to detoxify staphylococcus toxin prepared by a modification of methods reported by Parker and by Dolman. Minute quantities of stock dye solution were added to filtrates and these were exposed on ice 20 cm. below a 100-watt lamp. Treatment removed the hemolytic, necrotizing and killing properties of the toxin but did not destroy the antigenicity of the product.—*M. S. Marshall.*

ONO, K. Studien über die Färbung der Spirochaete pallidae, insbesondere über den Zusammenhang der Färbung mit den Vorbehandlungen. I. Einfluss der Vorbehandlung mit verschiedenen Fixierungs- und Beizmitteln auf die Färbbarkeit der Spirochaete pallidae und der Blutelemente. *Lues, Bull. Soc. Japon. Syphiligr.*, 11, 71-97. 1934.

The most satisfactory technic for staining the spirochaetes of syphilis in frozen sections is stated to be the following: Fix the tissue 90 min. to 24 hr. in 10% formalin, wash in tap water and cut. Transfer sections to a slide, dry at room temperature, fix again for 15 min. in the same strength formalin, wash thoroughly, treat 15 min. in 1% NaHCO₃, and wash thoroughly again. Stain 5-15 min. with 0.2-0.5% solutions of any one of the following dyes: Crystal violet, dahlia, extra violet, gentian violet, methyl violet, marine blue, or Victoria blue. (Cited from *Zentbl. Bakt.*, I Ref., 120, 345. 1936.)—*J. A. de Tomasi.*

ONO, K. Studien über die Färbung der Spirochaetae pallidae, insbesondere über den Zusammenhang der Färbung mit den Vorbehandlungen. III. Die Färbemethoden der Spirochaete pallidae. *Lues, Bull. Soc. Japon. Syphiligr.*, 11, 247-62. 1934.

Smear preparations of *Spirochaeta pallida* were fixed 15 min. with 10% formalin or any of the 3 preferred fixatives mentioned in paper No. 1 of this series, washed thoroughly in tap water and fixed another 15 min. in either 3% H₂O₂ or 1% KMnO₄. Various stains were tested by staining for 2-10 min. with 0.2-1% aq. solutions. Particularly good staining was obtained when the formalin fixation was followed by washing, soaking for 5-15 min. in 1% NaHCO₃, and washing again. The following dyes gave the best results: 1% ethyl violet (5 min.), 1% crystal violet (15 min.), 1% dahlia (15 min.), 0.2-1% gentian violet (2-15 min.), 0.2-1% sky blue (2-15 min.), 0.2-1% marine blue (2-15 min.), 0.2-1% methyl violet (2-15 min.), and 0.2-1% Victoria blue (2-15 min.). (Cited from *Zentbl. Bakt.*, I Ref., 120, 345-6. 1936.)—*J. A. de Tomasi.*

ONO, K. **Färbung der Spirochaetae pallidae in Gefrierschnitten.** *Lues, Bull. Soc. Japon. Syphiligr.*, 12, 82-5. 1935.

This is the first of a new series of studies on the *Spirochaeta pallida*. The first series, dealing with the recurrent spirochaetes, was reviewed in this journal. The author studied the fixing and mordanting properties of 20 solutions and the staining properties of 31 dyes. It is shown that the staining is largely dependent upon the pre-treatment of the slide. Of all fixing and mordanting solutions, 10% formalin, 3% H_2O_2 , and 1% $KMnO_4$ are preferred because the staining intensity of blood serum, proteins and erythrocytes is greatly reduced, assuring a strongly selective stain for the spirochaetes. Good results are also obtained with: 1:1 alcohol-ether, 5% alum, 5% $(NH_4)_2MoO_4$, 5% $K_2Cr_2O_7$, Müller's and Orth's mixtures. (Cited from Zentbl. Bakt., 1 Ref., 120, 346. 1936.)—J. A. de Tomasi.

SCHMIDT, W. **Versuche zur Färbung von Virusarten mit Viktoriablau.** *Zentbl. Bakt., 1 Abt. Orig.*, 136, 200-3. 1936.

Victoria blue 4R, a dye of the triphenylmethane series, has been used successfully by Herzberg (Zentbl. Bakt., 1 Abt. Orig., 130, 183-326, 1933-34; 131, 358, 1934) for staining virus preparations. In the case of smallpox, contact smears from infected cornea, testis, spleen, liver, kidney, and skin of rabbits and guinea pigs show elementary bodies distinctly present in great number. With the herpes virus, preparations from the cornea reveal light blue to violet bodies or particles, smaller than these elementary bodies but not so well defined. Negative results are recorded with vesicular stomatitis, foot and mouth, and fowl plague viruses. It is argued that these failures might be due to the much smaller size of the virus particles.—J. A. de Tomasi.

VERONA, O. **Manière de se comporter des microorganismes vis-à-vis de certaines substances colorantes. Étude particulière sur le vert malachite sur son application éventuelle en phytothérapie.** *Boll. Sez. Ital. Int. Micro.*, 7, 426-8. 1935.

A list of 26 common dyes including a few acid-base indicators were tested as to the bacteriostatic effects upon a large collection of microorganisms including bacteria, yeasts and molds, in dilutions ranging from 1:1,000 to 1:1,000,000. Even at the highest concentration the action of most of them proved to be very weak. The only exceptions were malachite green and, in a lesser degree, brilliant green and gentian violet. Germination of seeds was neither retarded nor stimulated by a 1:10,000 malachite green medium; but it seemed to be an obstacle to the further development of plants. It also proved to be a better fungicide than $CuSO_4$ or lime on wheat seed. Alkalies, such as KOH, convert it into the leuco-base which appears to be a stronger disinfectant than the original dye.—J. A. de Tomasi.

WALKER, A. W. **Comparison of precipitating action of basic dyes on bacteriophage and bacterial proteolytic enzymes.** *Proc. Soc. Exp. Biol. and Med.*, 34, 726-8. 1936.

Precipitation of the bacteriophage and proteolytic enzyme was accomplished by adding, to 1 cc. of anti-coli phage in synthetic medium and to 1 cc. of the filtrate from a 4-day culture of *Pseudomonas pyocyanea* in synthetic broth, 7 cc. of saline and 2 cc. of M/70 dilutions of various dyes. The mixtures were allowed to stand 18 hr. at room temp. No appreciable inhibiting effect was noted with any dye, except pyronin. The active agents were present in precipitates but not filtrates prepared with safranin, neutral red, Janus green, thionin, methylene green and toluidine blue. They were present in both precipitates and filtrates prepared with brilliant cresyl blue, cresyl violet, gallocyanin, pyronin and malachite green.—M. S. Marshall.

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STAIN TECHNOLOGY

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THE PRODUCTION OF BASIC FUCHSIN SUITABLE FOR THE FEULGEN TECHNIC¹

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*Contribution No. 268 from the Industrial Farm Products Research
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ABSTRACT.—Much difficulty has been experienced in obtaining basic fuchsins satisfactory for the Feulgen technic. A method of purification employing sulfur dioxide is described which has been found to improve many unsatisfactory fuchsins. It is also shown that if the best grade of commercial pararosanilin base is used and proper precautions observed in converting it to the chloride or the acetate, a product can be obtained which gives excellent results in the Feulgen technic. The methods by which the base can be converted to the chloride or acetate are described in detail. Precautions are given for avoiding, in the conversion of the base to the dye salts, contamination of the product with the impurities which interfere with the staining. The use of the acetate is recommended because of its greater solubility.

One of the principal obstacles to the successful application of the Feulgen technic has been the difficulty experienced in obtaining basic fuchsin (basic magenta) which could be satisfactorily decolorized and which would give proper tinting of the chromatin. Most of the trouble seems to be caused by a water soluble brown or yellow substance, of unknown constitution, which is not decolorized by sulfur dioxide and which, by diffuse staining, prevents or obscures the selective staining action of the fuchsin-sulfurous acid compound. The quantity of this undesirable material present can be roughly

¹This work was done in collaboration with Dr. H. J. Conn, Chairman of the Commission on the Standardization of Biological Stains. All the staining tests were carried out under his supervision in his laboratory at Geneva, N. Y., and that part of the work has been reported in detail by J. A. de Tomasi (Improving the technic of the Feulgen stain. *Stain Techn.*, 11, 137-44, 1936).

estimated by decolorizing a sample of the fuchsin according to the directions given under the heading "Preparation of Fuchsin Solution" in the Feulgen procedure recommended by de Tomasi.² When treated as described in the above-mentioned procedure the best samples give an absolutely colorless solution, but those which give a pale yellow or even a straw-colored solution usually prove quite satisfactory. The poorer samples of fuchsin decolorize to golden yellow, brownish yellow or dark brown solutions; the deeper the color the less satisfactory the staining will be. In some of these poorer samples a finely divided, dark-colored sediment can be observed. (Precipitation may also occur in a good sample if the bisulfite is added before the acid.) If the red color of the fuchsin is not completely discharged, after the expiration of the time specified, it usually indicates that a sufficient quantity of sulfur dioxide has not been supplied, most frequently because of deterioration of the bisulfite. (Insufficient acid may also prevent complete decolorization.) If the bisulfite is added in solution, the latter should be freshly prepared. Potassium metabisulfite ($K_2S_2O_5$) is to be preferred to the sodium bisulfites ($NaHSO_3$ and $Na_2S_2O_5$) as a source of sulfur dioxide because it is a more stable and uniform compound.

DISCUSSION OF RESULTS

In the first attempts to remove the interfering impurities, a method involving the use of an aqueous solution of sulfur dioxide as a solvent was employed. This method is described in a book by Fierz-David³ but is not mentioned elsewhere in the literature as far as we can determine. This method, given in detail below, was chosen because of its obvious relationship to the decolorization procedure of the Feulgen technic. It was applied to a series of commercial fuchsin samples from various sources which were known to be unsatisfactory for use in the Feulgen technic. Some were biological stains for which certification had been refused for one reason or another. The results are given in Table 1.

The results were encouraging and it can be seen that while this method of purification is not of universal application, it has considerable value in many cases. In every case there was improvement and in one case (Sample No. 9) a sample previously classified as only fair for the Feulgen technic was converted by this procedure to one of the best ever obtained.

²See paper cited in footnote 1.

³Fierz-David, H. E. 1926. *Kunstliche Organische Farbstoffe*. Julius Springer. Berlin. (See p. 247.)

The next step was to synthesize in the laboratory the four lowest fuchsin homologs⁴ and to incorporate a slightly modified form of the above sulfur dioxide purification as a step in the regular method of preparation. The results were disappointing. The products were improved by this method of purification but were still highly unsatisfactory for the Feulgen technic, so this phase of the work was abandoned.

TABLE 1. EFFECT OF SULFUR DIOXIDE PURIFICATION UPON FUCHSIN SAMPLES PREVIOUSLY FOUND UNSATISFACTORY

Sample No	Character of Original	Method of Precipitation	Behavior in the Feulgen Technic*	
			Before Purification	After Purification
1	Textile dye	Crystallized from H ₂ O	Unsatisfactory	Distinctly improved
2	Textile dye	Recrystallized from C ₂ H ₅ OH	Unsatisfactory	Distinctly improved
3	Biolog. stain	Crystallized from H ₂ O	Unsatisfactory	Somewhat improved
4	Biolog. stain	Crystallized from H ₂ O	Unsatisfactory	Improved, but stained weakly
5	Biolog. stain	Crystallized from H ₂ O	Unsatisfactory	Improved, but stained weakly
6	Biolog. stain	Crystallized from H ₂ O	Very poor	Improved, stained strongly but not selective
7	Biolog. stain	Crystallized from H ₂ O	Unsatisfactory	Excellent decolorization, staining fair
8	Biolog. stain	Crystallized from H ₂ O	Unsatisfactory	Good decolorization, staining fair
9	Biolog. stain	Crystallized from H ₂ O	Fair	Decolorization almost perfect, staining good

*Tests made at Stain Commission laboratory, Geneva, N. Y.

It was then decided to work out a purification method using as a starting material a representative commercial product, one which would always be readily available on the market and would be most likely to be uniform in quality from batch to batch. Furthermore,

⁴Scanlan, John T. The magenta series. I. The preparation and spectrophotometric study of the lower basic members. *J. Amer. Chem. Soc.*, **57**, 887-92. 1935.

this product would have to be available as the acetate as well as the chloride, since the former is often preferred because of its greater solubility. The acetate, however, is not a common commercial product and it is the custom of at least one stain distributor to purchase the carbinol base and convert it to the chloride and the acetate. In consideration of all the above factors, the product chosen was the best grade of pararosanilin base, which is especially prepared for use in the preparation of the greenest brands of spirit blues and soluble

TABLE 2. EFFECT OF VARIOUS METHODS OF TREATMENT APPLIED TO A SAMPLE OF THE BEST GRADE OF COMMERCIAL PARAROSANILIN BASE

	Sample No.	Method of Treatment			Behavior in Feulgen Technic*	
		Purification	Salt Prepared	Method of Precipitation	Appearance of Decolorized Solution	Staining
	10	With SO_2	Chloride	Crystallized from H_2O	Very slightly yellow	Good
	11	With SO_2	Chloride	Salted out with NaCl	Very slightly yellow	Good
	12	None	Chloride	Crystallized from H_2O	Slightly yellow	Good
	13	None	Acetate	{ Salted out with correct proportion of sodium acetate	Colorless	Excellent
	14	None	Acetate	Evaporated to dryness	Golden yellow, trace dark sediment	Very unsatisfactory
	15	None	Chloride	Evaporated to dryness	Brownish yellow, trace dark sediment	Very unsatisfactory
	16	None	Acetate	{ Salted out with too much sodium acetate	Golden yellow	Fair

*Tests made at Stain Commission laboratory, Geneva, N. Y.

blues (the triphenylated pararosanilins). We believe that the process of preparing this particular grade has been developed to the point where a product equal in quality to that with which we worked can always be obtained. It must be emphasized, however, that grades of rosanilin bases other than the particular one specified above will probably not be equally satisfactory. What we believe to be a representative sample of this best grade of pararosanilin base was obtained from the Calco Chemical Company thru the courtesy of Dr.

M. L. Crossley. This base was converted by various methods to the chloride and the acetate with and without purification by the sulfur dioxide method. The results of these experiments are given in Table 2.

It will be seen that the purification with sulfur dioxide made little or no improvement in the quality of this particular batch. But it will also be observed that by certain methods of conversion to the dye salts this excellent sample can be made absolutely worthless for application in the Feulgen technic. Apparently even the best samples of pararosanilin base contain some of the brown impurity already referred to, and this must be eliminated when the salts are prepared. To obtain a product, therefore, free from this impurity the solutions should not be evaporated to dryness. Such a procedure was followed with Samples No. 14 and No. 15 with the deleterious effects indicated in Table 2. In the case of pararosanilin chloride, the best procedure is to allow it to crystallize from a dilute acid solution. No particularly bad effects were observed when the chloride was salted out with sodium chloride (this step would be necessary with the more soluble higher homologs) but if too much sodium chloride should be used it is probable that the brown impurity, which remains in solution in the ordinary crystallization, would also be thrown out. Since the acetate is too soluble in water to crystallize from dilute solution, it was necessary to salt it out with sodium acetate. It was found that only a very small quantity of sodium acetate was required and by carefully regulating the quantity added, most of the pararosanilin acetate could be precipitated while the brown impurity remained entirely in solution. The product so obtained was extremely easy to handle during and subsequent to the filtration, washing and drying. When decolorized with potassium metabisulfite according to the procedure already referred to, it gave an absolutely colorless solution and had excellent staining properties; sample No. 13, in fact, was one of the best ever examined in the Feulgen stain. The quantity of sodium acetate used must be limited very carefully, however, as too much sodium acetate precipitates the brown impurity also, as shown by Sample No. 16. The maximum quantity which could be added without causing precipitation of the impurities was determined for this batch and is given in the directions for the preparation of Sample No. 13. Whether these directions will apply equally well to subsequent batches is questionable, but the correct quantity can be determined in any case by precipitation from aliquot portions with varying quantities of sodium acetate and the application of the decolorization test to the products obtained.

CONCLUSIONS

It is believed that a fuchsin satisfactory for use in the Feulgen reaction can be obtained by starting with the best grade of commercial pararosanilin base and preparing from it, according to the directions given below, either the chloride or the acetate. The use of the acetate rather than the chloride is recommended because its greater solubility makes it possible to keep in solution at room temperature the concentration of dye required in the Feulgen solution.

In many cases an unsatisfactory batch of fuchsin can be improved sufficiently to give very good results by purification with sulfur dioxide according to either of the two methods given below. In some cases the use of decolorizing charcoal (Darco, for example) is helpful.

EXPERIMENTAL

PURIFICATION OF FUCHSIN WITH SULFUR DIOXIDE

Method 1. Applicable when dye is in the form of salt.⁵ Samples No. 1 to No. 9 inclusive (Table 1).

A paste was made of 10 g. of the dye and 40 cc. of water and into this was stirred 0.5 g. of sodium bicarbonate. Gaseous sulfur dioxide was passed thru this mixture for about 45 min. and the undissolved residue was then removed by filtration and washed with about 50 cc. of water. To the combined filtrate and washings were added about 150 cc. of water and 1 cc. of concentrated hydrochloric acid and the mixture was boiled gently for an hour to expel the sulfur dioxide. It was then diluted to about 1500 cc. with hot water, heated to boiling, then allowed to cool and stand at room temperature over night. The crystallized dye was filtered off and air dried at room temperature.

Method 2. Applicable when the dye is in the form of the carbinol base. Sample No. 10.

The dry base⁶ was finely ground in a mortar and a 32 g. portion was suspended in about 250 cc. of water and stirred mechanically for a short time. Sulfur dioxide was then bubbled slowly thru the suspension with continuous stirring. After about 45 min. no further solution seemed to be accomplished altho the procedure was continued about 2 hours longer. In one experiment a smaller volume (150 cc.) of water was used but no effect was noticed except a reduction in the yield. The small quantity of material which remained undissolved was filtered off, the filtrate was diluted with 750 cc. of water to which 14 cc. of concentrated hydrochloric acid had been added, and boiled gently with addition of hot water to keep the volume constant, until all of the sulfur dioxide was expelled

⁵See footnote 3.

⁶The base need not be dried if this purification is to be applied immediately after the base is precipitated. After it is filtered and washed it can be suspended in the proportion of water herein specified and treated with sulfur dioxide as described. In fact this procedure seems advantageous as the base dissolves much more rapidly if it has not been permitted to dry out.

The solution was allowed to cool and stand at room temperature for 72 hrs. The crystallized dye was filtered off and air dried. Yield 22 grams.

Sample No. 11.

An equally good product was obtained by following the same procedure up to the point where the sulfur dioxide was completely expelled and then adding 200 cc. of hot saturated sodium chloride solution to the boiling dye solution with vigorous stirring. The solution was allowed to cool and stand at room temperature for several days. The product was filtered off and air dried. Yield 29 grams.

If the acetate is desired instead of the chloride we believe it would be feasible, altho it has not yet been done, to modify the above procedure so that acetic acid could be substituted for hydrochloric and the acetate salted out as described below.

METHODS RECOMMENDED FOR PREPARING THE DYE SALTS FROM BASES WHICH DO NOT REQUIRE PURIFICATION

Conversion of pararosanilin base to the chloride. Sample No. 12.

A 16-gram portion of pararosanilin base was added to a hot solution of 15 cc. of 6N hydrochloric acid in 750 cc. of water, the mixture was stirred well and boiled for about an hour. It was then allowed to cool and stand at room temperature for several days, chilled in an ice-bath and the crystalline product filtered off and air dried at room temperature. Yield 14 grams.

Conversion of pararosanilin base to the acetate. Sample No. 13.

A solution of 4 cc. of glacial acetic acid in 200 cc. of water was heated to steam-bath temperature and poured over 10 g. of pararosanilin base. The mixture was digested on the steam bath for about half an hour with occasional stirring and allowed to cool and stand over night. It was then filtered cold to remove a small quantity of dark-colored, insoluble material. The volume of the filtrate was made up to 200 cc. with cold water and 10 cc. of sodium acetate solution, containing 384 g. of $\text{NaC}_2\text{H}_3\text{O}_2$ per liter, were added with vigorous stirring. The mixture was allowed to stand several days at room temperature, then filtered and the product was washed with about 75 cc. of water containing approximately the same concentration of sodium acetate as the dye solution. The product filtered easily and dried quickly at room temperature. It consisted of dark green, dense, microscopic tetrahedral prisms, very easy to handle. It proved one of the best samples ever examined for the Feulgen stain. Yield 8 grams.

EXPERIMENTS SHOWING THE BAD EFFECTS OF CERTAIN METHODS OF PREPARING THE DYE SALTS

Samples No. 14 and No. 15.

In these experiments the base was dissolved in water containing 10% excess of hydrochloric and acetic acid, respectively, and the solution was evaporated to dryness.

Since this method of procedure does not provide for the elimination of the brown impurities, which seem to be present in even the best samples of the base, the products were found to be very unsatisfactory and this method of conversion should be avoided.

Sample No. 16.

The procedure followed in this experiment was exactly the same as that described for Sample No. 13 except that the final volume of the solution was about 25% less and the quantity of sodium acetate used to precipitate the dye was about 10 times as great. The result was the precipitation with the dye of the undesirable brown impurities which in Sample No. 13 remained in solution and were eliminated. The product in this case was much less satisfactory than No. 13. In salting out the acetate the use of too much sodium acetate must, therefore, be carefully avoided.

AN IMPROVED METHOD FOR THE STUDY OF CHROMOSOMES

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ABSTRACT.—It has been felt strongly that the current methods for the fixation of chromosomes are inadequate, inasmuch as the reagents are unduly long in reaching the important elements of the cells, particularly the nucleus. A realization of this situation has been at the bottom of the development of the so-called smear method. This method can, however, be advantageously employed only where loose cells are available, as in the reproductive elements of plants and animals. The present author has found that cutting up the material in very thin slices of almost microscopical tenuity, and passing them immediately into the best fixing solutions, namely those containing osmic acid, gives excellent results. The material is then assembled on cards in accordance with the present author's mass method (for which see the original article), and after embedding in nitrocellulose is sectioned 5μ or even thinner. Practically all methods of staining may be used, but the most satisfactory results have been achieved with Heidenhain's hematoxylin bleached almost to disappearance and followed by prolonged treatment with an aqueous safranin. This differentiates the chromosomal structures extremely well. It is clear by this method that chromosomes in all cases consist of a ground substance in which are situated two chromatids. In the somatic tissues these chromatids are apparently generally spiral and run in opposite directions. The crossing points of these spirals are responsible for the optical illusion which has been designated *the gene*.

The investigation of the minute structure of chromosomes rightly occupies a large place in cytological work at the present time. It has been increasingly realized that the immediate access to the cells of the best possible reagents, such as osmic acid and some of its combinations, is of the utmost importance. Out of this realization has grown the smear method, which as such is applicable only to the mother cells of the reproductive cells proper at an advanced stage after they have become separated from one another. In the case of plants it does not in general prove successful, in the later stages, where the gametophytes are in process of development. Further disadvantages are the difficulties of fixation of the prophases of meiosis and the danger of mechanical injury to the cells presenting the stages of meiosis. Still another disadvantage is the frequent loss of a large number of preparations in the process of staining and mounting.

Convinced that some method should be found to obviate these disadvantages and at the same time to extend improved methods of fixation to the somatic cells of plants and animals, which are not really available on any extensive scale, the author has given much time and effort during the past three years to the study of this important technical problem. As the result of many trials extending over a wide range of plants and representative animals, a surprisingly simple solution has been reached which makes possible the securing of numerous preparations, exhibiting good preservation of the minute but important structural details of chromosomes. Since these results may be of interest to fellow workers an account of them is supplied in the following paragraphs:

The material is cut free hand into very thin slices, almost thin enough for microscopic study (about 0.5 mm. or less). In the case of young inflorescences, transverse slices are made across the whole flowers in various stages of development, which are immediately dropped into the selected preserving fluid as rapidly as made. Naturally the flowers and parts of flowers tend to separate from one another during the operation, but as will be seen in the sequel this is of no disadvantage. In the case of ovaries of plants, slices are made in the same manner. This holds true also for the reproductive glands of animals. In the case of the skin and mucous membranes, a piece is rapidly cut out and finely scored with a very sharp knife on a substratum of cork. In the case of the roots of plants, the tips are placed between thumb and index finger after the manner of the older botanists and sliced longitudinally. Small roots require only a single cut while larger ones may be scored several times.

At short intervals the air is expelled from the material by the use of a pump which, in the laboratory, may be electrical. The water pump is scarcely powerful enough to remove the air from the tissues sufficiently rapidly to insure perfect fixation. In the field, a reversed automobile pump, in which the piston is modified so as to exhaust air and gases in the upward stroke, is most useful. If the larger automobile pumps are used (1¾ or 2 inch barrel) no valves are necessary in the course of the tube connecting the pump with the bottle of material which is being preserved. Frequent pumpings should be made during the course of fixation to insure rapid penetration of all the objects. Three to a dozen strokes are all that are necessary, in other words, enough to cause the material to sink rapidly to the bottom of the preserving fluid. It is often advantageous to keep the material in a refrigerator during the process of hardening.

For the more homogeneous tissues, particularly the somatic ones,

Benda's fluid and Champy's have proved very satisfactory on account of the absence of swelling. In the case of inflorescences it is sometimes advantageous to use an alcoholic medium, e.g., Zacharias' fluid (4 parts absolute alcohol, with 1 part to $\frac{1}{8}$ part glacial acetic acid, to which a few drops of 2% osmic acid solution are added). Blackening with osmic acid which is characteristic of all these fluids is of no disadvantage as it can readily be removed by the use of hydrogen peroxide at the time the sections are made.

The successful utilization of the procedure described above virtually necessitates the writer's mass method in preparing for sectioning.¹ After being gradually brought into strong alcohol (95%) the mass of preserved material is sorted under a low power of the dissecting microscope to remove parts which are of no interest. After concentration, in case this is desirable, the material is transferred for half an hour or more to a mixture of equal parts of alcohol and commercial glycerin. In the meantime a number of cards are prepared by cutting up homogeneous cardboard into pieces 18 x 25 mm. The cardboard should be not less than a millimeter thick and *without any paper pasted to either surface*. The material can either be laid down in regular rows on the pieces of cardboard, which are previously soaked in glycerin and alcohol or merely spread out smoothly over the surface with a scalpel or section lifter. The cards are then flooded gently by means of a rod or dropper with 6% nitrocellulose solution in ether and alcohol. Meanwhile pieces of paraffined paper have been cut of the same size as the cards and over each one of the latter a piece of paper is placed so as to cover the material to be embedded. Then the cards are covered (conveniently in threes) by an ordinary microscopic slide, on top of which is placed a moderate weight the heaviness of which is governed by the delicacy of the material. After 10 or 15 minutes, the slide is removed and the paraffined papers are secured to the cards by wrappings of thin thread (No. 50 or 60) in two directions, which are tied securely. The cards are now put in 95% alcohol to which a little chloroform has been added. In half an hour they are ready for the next stage. In a watch glass containing alcohol the papers are pricked at intervals of a millimeter or less with a fine sewing needle (No. 12) sunk in a small cork. The cards are now transferred to alcohol, which must be changed to remove the chloroform. They are then placed under the air pump until all air is removed.

After thoro dehydration by 2 or 3 changes of absolute alcohol, the cards are embedded in nitrocellulose. For a description of this method and the author's subsequent improvements of it, the reader

¹Jeffrey, E. C. 1928. Technical contributions. Bot. Gazette, 86, No. 4, 456-467.

is referred to the articles by Jeffrey¹ and Wetmore². For animal tissues the article by Walls³ may be consulted with advantage.

The sectioning is to be done with a sliding microtome. It is highly advantageous to have the sections no thicker than 5 to 10 μ . They should be in all cases bleached with hydrogen peroxide. Merck's Superoxol, which has a strength of 30%, is most useful. This is reduced to from 4 to 10% by dilution with alcohol of from 95 to 70%. The bleaching may be carried on over night in the weaker percentages of hydrogen peroxide but with the stronger ones a few hours are sufficient. Heidenhain's hematoxylin gives very clear images, particularly if the hematoxylin is almost bleached out with iron alum and is followed by weak aqueous safranin over night. This is largely removed in the process of dehydration but enough remains in the nuclear structures to support a richness of detail not attained by hematoxylin alone.

With the technical manipulation described above, the active somatic chromosomes are seen to consist of two oppositely revolving spirals, the chromatids. The crossing of the opposed turns of the spirals is clearly the basis of the beaded appearance of the spireme and chromosome described by many authors. The structural features referred to are present in all chromosomes and spiremes if the procedure has been carefully used. The method permits the comparison of all active nuclei, whether reproductive or somatic. No advantage has been found to result from the use of fumes of ammonia, nitric acid, etc., as recommended by a number of recent authors.

In all cases examined by the author the spirals of the chromosomes of the sporophytic generation have their so-called gyres crossing in opposite directions. This is true even of *Trillium* and *Tradescantia*, in which the chromosomes of the first and second meiotic divisions have two chromatids in which the spirals are parallel and not in opposition as is apparently generally the case for somatic tissues.

The present account is supplied in the belief that the method featured will make possible a much needed clarification of the nuclear phenomena not only of meiosis but also of mitosis. It may further be used advantageously on the nuclear divisions of the gametophytes of the higher plants. It appears to present definite advantages over the smear method so much in vogue at the present time, due to its much wider applicability and in the absence of mechanical injury to the nuclear elements. It further makes it possible to follow with precision all stages of the nucleus, whether resting or in activity.

¹Wetmore, R. H. 1932. The use of celloidin in botanical technic. *Stain Techn.*, 7, 57-62.

²Walls, G. L. 1932. The hot celloidin technic for animal tissue. *Stain Techn.*, 7, 135-45.

A METHOD FOR GROWING AND FOR MAKING PERMANENT SLIDES OF POLLEN TUBES

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In a study of the nuclear division of the pollen tube and of the self-sterility phenomenon in the California poppy, *Eschscholzia californica* Cham., a method was devised for growing, fixing, and staining pollen tubes. This method provides an easy way of measuring the growth rate of the pollen tubes and a short procedure for making permanent slides of them.

A 1.5% agar solution is prepared and to this is added a quantity of cane sugar. The optimum amount of sugar varies with the material used: *Eschscholzia*, 5%; *Papaver* (poppy), 3%. The agar solution is kept near the boiling point. A small drop of egg albumen is placed on a slide, and after it is smeared over the surface as much as possible is wiped off. By using a small brush a very thin layer of the agar solution is put on the slide. The slide is then placed on a piece of moistened paper toweling or filter paper in a covered Petri dish. As soon as the agar is cool the pollen may be dusted upon it.

The pollen will begin to germinate within a few minutes after being placed on the agar. In a few hours, the time varying with the material used, the slide is placed face down in a dish of fixing fluid and left for 15 minutes. Navashin's fixative gives good results. After fixation the material is washed and stained with crystal violet in sat. aq. solution (certified lots CC-6 and CC-7 employed by writer); the same procedure used in making permanent pollen mother-cell smears is followed. The agar loses the stain rapidly so that the finished slide is clear, or at the most it has only a light bluish cast. By regulating the time intervals for fixing the material the different stages of nuclear division can be obtained.

Other fixatives and stains, including aceto carmine, have been used. Altho most of them gave satisfactory results, fixation in Navashin's followed by crystal violet stain is recommended.

The growth rate of pollen tubes can be measured if one prepares a culture of pollen as described. In some materials, such as *Eschscholzia*, in which the pollen tubes explode when brought in contact with the air, it is necessary to enclose the pollen in an air tight chamber. The following method provides an easy way for making such a chamber, and also facilitates the measuring of pollen-tube growth.

Take a piece of thin cardboard the size of a slide and cut out two connecting compartments as shown in Fig. 1. Impregnate this piece of cardboard with hot paraffin. Warm a slide and then place the paraffined cardboard on it. As soon as it sets, the albumen and the agar solution can be placed in the large chamber. Within the small one place a piece of moistened filter paper. The amount of moisture needed depends upon the species of plant used. The pollen is dusted upon the agar as nearly as possible in a straight line across the slide. A thin layer of egg albumen is placed on another slide.

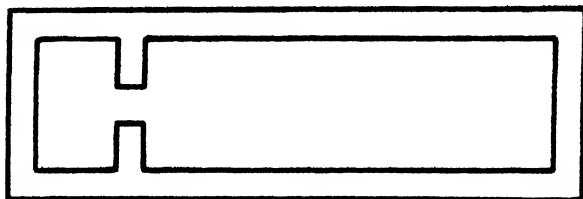


Fig. 1. To illustrate shape in which cardboard is cut

This second slide is warmed and placed with its albumenized side down on the cardboard. The albumen prevents the moisture from collecting in droplets on the slide. The culture can be placed in a temperature chamber and examined as often as desired without bringing the pollen tubes in contact with the air.

A culture prepared in this way can only be studied under a low power objective. A higher power objective may be used if one uses a thinner piece of cardboard and a long cover glass in place of the top slide.

These two methods may be advantageously combined to give a more accurate means for determining the fixing periods, since the length of the pollen tube affords a good index of the stage of nuclear division.

THE USE OF LEUCO TRIPHENYLMETHANES AS REAGENTS FOR BACTERIAL POLYSACCHARIDES. (PRELIMINARY REPORT)

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ABSTRACT.—The present investigation was initiated because of the need of a reagent for detecting and estimating bacterial polysaccharides without having to hydrolyze them before testing. It was found that acidulated solutions of leuco triphenylmethanes and reduced bases of triphenylmethanes are either precipitated or oxidized to colored compounds by bacterial polysaccharides. Non-bacterial polysaccharides and simpler carbohydrates gave negative reactions. The wide variety of reactions obtainable by using different compounds of this group of intermediates makes it possible to apply them to many different polysaccharide problems. An outline is given of the reactions obtained by typical reagents.

Qualitative and quantitative tests of bacterial polysaccharides depend upon complex chemical procedures. In this paper will be described the preliminary development of simple colorimetric and turbidimetric reactions (Chapman, 1936a) which are adaptable to crude extracts or even dense and pigmented bacterial suspensions. The only requirements are having a slightly acid extract or suspension and avoiding certain interfering substances (peptones, oxidizing agents).

While studying the reaction of crystal violet and related compounds with staphylococci, it was observed that when an acidulated solution of "leuco" methyl violet was added to suspensions of staphylococci, deep blue or violet colors were produced. When the reagent was added to distilled water no color developed within 1 hour. Further investigation revealed that the active ingredient of the bacterial suspension was a polysaccharide. Other bacterial polysaccharides, such as those from streptococci and colon bacilli, also gave positive results but no reactions were obtained with numerous laboratory sugars, starch and dextrin. Commercial peptones and, as was to be expected, oxidizing agents affected the reagents.

Because of the possible value of this reaction in the study of bacterial polysaccharides the subject was investigated further, using staphylococcal polysaccharides.

In addition to "leuco" methyl violet, other leuco triphenylmethanes

also reacted with staphylococcal polysaccharides, giving either white or colored precipitates. Leuco diphenylmethanes, such as leuco auramin, however, did not react. This suggests that the power to react with bacterial polysaccharides is confined to the leuco triphenylmethanes.

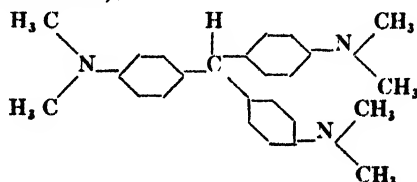
A number of "leuco" compounds are on the market but their composition is not uniform. Most of them appear to have been prepared by reduction of the corresponding base of the dye and contain considerable inert material, probably derived from the reducing agent. Solutions of this type of "leuco" compound are considerably colored. Another type, produced synthetically by the Eastman Kodak Company, appears to be chemically pure and its solutions are colorless or only faintly colored. Because "leuco" implies "colorless", the term will be applied to such compounds and the reduced dye bases will be referred to as "reduced bases".

An occasional compound was oxidized so rapidly, even with distilled water, that it was unsuitable for these studies.

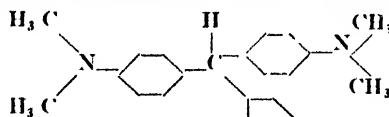
A solution of each compound was prepared by dissolving 0.50 g. of the powder (or paste, as in the case of reduced brilliant green) in 100 cc. of distilled water by the aid of a minimum quantity of N/1 HCl. The exact amount of HCl was critical and determined the sensitivity of the reagent. Reduced methyl violet base and a few other reduced bases were only partly soluble, even with a large excess of HCl, and it was necessary to choose an amount of HCl which gave best color development with the polysaccharide solution and a minimum color in the distilled water blank. Other amounts of HCl gave either slow color development or highly colored blanks (due to excessive auto-oxidation). With precipitating reagents, an excess of HCl prevented complete precipitation of the polysaccharide, while insufficient HCl gave a turbid blank due to precipitation of the reagent in the neutral solution.

More exact information about these reagents and their reactions with bacterial polysaccharides must await the preparation of larger quantities of polysaccharides than were available when this work was in progress.

More intensive study was made of leuco crystal violet (hexamethyl triamino triphenylmethane),



leuco malachite green (tetramethyl diamino triphenylmethane),



and its derivatives containing the following radicals in place of the unsubstituted phenyl group in the above formula:

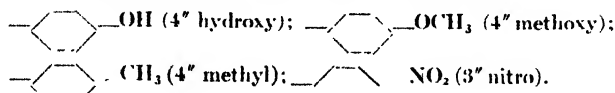


Table 1 illustrates the findings with this group of reagents. With the exception of the methyl and methoxy derivatives of leuco malachite green, dense precipitates were obtained with staphylococcal polysaccharides. The relative stability of the leuco crystal violet, leuco malachite green and derived reagents makes them useful for both qualitative and quantitative tests, but the solutions to be tested must be clear.

TABLE 1. CHARACTERISTICS OF LEUCO TRIPHENYLMETHANES AND REDUCED TRIPHENYLMETHANE BASES, AND THEIR REACTIONS WITH STAPHYLOCOCCAL POLYSACCHARIDES

Compound	Physical State	Color of Solution	Amount of ppt.	Color of ppt.	Remarks
Leuco crystal violet	Silver needles	Pale green, changing to pale violet	Dense	White, becoming violet*	Reagent very sensitive. Solution relatively stable.
Leuco malachite green	White amorphous	Colorless	Dense	White, green in 2 weeks	
Leuco nitro derivative	Greenish-yellow	Pale green	Dense	Yellow	
Leuco methyl derivative	Gray	Oxidized too rapidly			Unsuitable for tests
Leuco hydroxy derivative	Violet	Brownish-green	Dense	Violet	
Leuco methoxy derivative	Grayish-white	Green	Slight	White	
Reduced methyl violet base	Violet crystals	Dark green	None	—	Intense bluish violet solution with polysaccharides.

*Many of the different precipitates tended to redissolve on standing.

Reduced methyl violet base did not precipitate the polysaccharides but produced highly colored compounds which were useful in the study of turbid solutions or bacterial suspensions because the violet reaction product stained the gross particles. When freshly prepared, this reagent produced a violet color almost immediately with a solution containing staphylococcal polysaccharide. After the reagent had stood for 1 or 2 days, the violet color took almost 1 hour to reach its maximum intensity. The reagent then retained this degree of sensitivity for several weeks when steps were taken to minimize the absorption of oxygen.

Reduced malachite green base acted similarly to leuco malachite green but the precipitate formed by reduced brilliant green base soon redissolved. Reduced magenta base oxidized too rapidly for testing.

DISCUSSION

Because of the possible importance of these reactions in the study of bacterial polysaccharides, this preliminary report has been presented without waiting for the results of more precise tests. Present investigations include: studies of the keeping qualities of the reagents; the relation between hydrogen-ion concentration and sensitivity; the optimum proportions of reagent and polysaccharide; the behavior of different polysaccharide fractions; and studies of the relation between chemical constitution of the reagent and its power to react with different bacterial polysaccharides.

By means of these reagents, it has been possible to work out a simple method for the isolation of large quantities of staphylococcal polysaccharides of a high degree of purity. Other uses for the reagents are obvious.

Previous findings (Chapman, 1936b) suggested a specific reaction between pentamethyl- and hexamethyl-pararosanilin and staphylococcal cultures. With leuco bases and reduced bases, the reaction is not quite so specific and seems to depend upon the oxidizing power of the polysaccharides.

The type of reaction with staphylococcal polysaccharides varied considerably. The leuco base of crystal violet produced a white precipitate which became colored after standing a few hours. Reduced methyl violet base did not precipitate the polysaccharides but produced highly colored solutions. The leuco base of malachite green gave a white precipitate which did not become colored until after standing several weeks. Derivatives of leuco malachite green gave either white or colored precipitates. The production of colored solutions is, therefore, characteristic of the reduced base of methyl violet.

With the crude methods available, the reagents were capable of detecting 0.01% of polysaccharide. It seems possible that, by adjustment of experimental conditions, an amount as small as 0.001% may be detected.

Because of the interfering action of peptones, the reactions are not applicable to crude broth cultures.

CONCLUSIONS

Solutions of leuco bases and reduced bases of certain triphenylmethanes, particularly those of penta- and hexa-methyl triamino triphenylmethane and tetramethyl diamino triphenylmethane and some of its derivatives react with staphylococcal and other bacterial polysaccharides.

The reaction products, which are either white or colored precipitates or colored solutions, appear to be useful for the detection and quantitative estimation of these polysaccharides.

REFERENCES

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CHAPMAN, G. H. 1936b. Specificity of the dye in the crystal violet agar reaction of staphylococci. *J. Bact.* **32**, 199.

A DIOXAN TECHNIC FOR TRIPLE STAINING

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Columbus, Ohio*

ABSTRACT.—The first part of the paper summarizes the ways in which technicians have used dioxan in the various phases of micro-technic. The rest of the paper consists of a detailed account of the use of dioxan in triple staining. Specific directions are given for modifications of Castroviejo's method and Mallory's triple connective tissue stain. Special emphasis is placed upon the fact that dioxan will not wash the stains out of the sections in the dehydrating process to any great extent. This makes triple staining easier.

In comparing various dehydrating agents, Baird (1936) concluded that dioxan causes the least amount of shrinkage and keeps the tissues from becoming hard and brittle.

McClung (1936) substituted dioxan thruout the entire micro-technic process, including staining. He states that dioxan speeds up the entire process and at the same time produces better results.

Guyer (1936) also mentions the use of dioxan in staining and employs a 0.10% solution of erythrosin as a counterstain with hematoxylin, because eosin is relatively insoluble in dioxan.

The writer finds that by applying heat to dioxan (an electric hot plate is the safest) or by adding a few drops of tertiary butyl alcohol, enough eosin will go into solution to stain slowly. The same result is obtained by allowing the dioxan and eosin powder to stand for several weeks, shaking it occasionally. By using eosin in this manner, it stains slowly enough to be observed and can be stopped when the desired intensity has been reached. This eliminates the possibility of overstaining, which is so common with the alcoholic method.

TRIPLE STAINING

The dioxan method is particularly useful in triple staining because the stains will not wash out to any great extent in dehydration. Two triple staining methods are outlined below:

1. Modification of Castroviejo (1932):

The success of this stain depends to a great extent upon the fixative used. The writer finds that the following modification of Petrunkevitch's cupric-paranitrophenol gives excellent results.

60% dioxan.....	100 cc.
Nitric acid (C. P. sp. gr. 1.41-1.42) i. e. 70%.....	3 cc.
Cupric nitrate (C. P. crystals).....	2 g.
Paranitrophenol (C. P. crystals).....	5 g.

STAIN TECHNOLOGY, VOL. 12, NO. 1, JANUARY, 1937

Let stand 2-3 days; shake it occasionally; filter and add 5 cc. of ether.

The tissues should be placed in the fixatives for 24 to 48 hours. Pieces of 2 to 4 mm. in size will be fixed in four to six hours. Such objects as tadpoles will become bleached and cleared if allowed to stand for about four days. After fixation the tissues may be dehydrated and embedded by the usual dioxan method and then sectioned. Place in xylene, remove to dioxan for a few minutes, and then into equal parts of dioxan and water. This is followed by water.

The stains used were acetic-fuchsin-formalin and picric-indigo-carmin. These stains were modified as follows:

Acetic-fuchsin formalin:

A stock solution of saturated alcoholic basic fuchsin was prepared.¹

From this stock, carbol fuchsin is made as follows:

Saturated alc. solution of basic fuchsin (Coleman and Bell)	30 cc.
5% solution of phenol (melted crystals)	270 cc.

From this carbol fuchsin, the acetic-fuchsin-formalin must be made fresh every day as follows:

Distilled water	150 cc.
Carbol fuchsin	15 cc.
Glacial acetic acid	1 cc.
Formalin	1 cc.

Picric-indigo-carmin:

Saturated aq. solution of picric acid	400 cc.
Indigo carmine (Merck)	1 g.
Glacial acetic acid	8 cc.

Stain in the acetic-fuchsin-formalin for five minutes.² Rinse in distilled water for not over two seconds,² and place in the picric-indigo-carmin for one and one-half minutes.² Rapidly rinse in distilled water for not over two seconds. Place in 50% dioxan for two seconds and in two changes of pure dioxan for 45 seconds each. Follow by xylene and mount in dammar.

The stain differentiates as follows:

Connective tissue	blue
Nuclei	lavender or red
Blood corpuscles	yellow
Muscle and cilia	yellowish-green

¹This solution should be allowed to stand for at least two weeks in order to insure complete saturation.

²This timing should be very accurate. It is best to use a stop watch. The timing varies slightly with the thickness of the sections.

2. Mallory's Triple Connective Tissue Stain:

In the use of this staining method, the important thing to keep in mind is the fact that the stains used are only slightly soluble in dioxan. Therefore, the time in which the slides are left in the stains should be greatly shortened since the excess stain will not wash out in the dehydrating process. In most instances the staining time is less than half of that usually required. This is also true for Krichesky's (1931) modification.

While any of the common fixatives, including Petrunkevitch's mentioned above, gives very good results, the writer finds that the following modification of Bouin's picro-formol is extremely satisfactory for this method.

Picric acid, sat. dioxan solution	75 parts
Formalin.	25 parts
Glacial acetic acid	5 parts

ADVANTAGES OF METHOD

1. A considerable amount of eosin can be dissolved in dioxan by the application of heat or the addition of a few drops of tertiary butyl alcohol or both. Allowing it to stand for several weeks also increases the amount that will go into solution. This causes the tissue to stain more slowly; therefore, the danger of over-staining is practically eliminated.

2. Triple staining, especially with Mallory's, is more easily accomplished since the stains will not wash out in water. The Castroviejo modification for triple staining is a little more difficult to use since the stains will wash out in dioxan but not nearly to the extent as with the alcohol method. Its advantages, however, are well worth the trouble since it is a very specific stain for the tissues mentioned in the body of this article.

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COOLING DEVICE FOR THE MICROTOME

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Temperature conditions often make it desirable to chill the blade and block when cutting paraffin sections. An inexpensive and convenient device for doing this can easily be made as follows.

A piece of sheet duraluminum, preferably 0.5-0.75 mm. in thickness, is cut according to the accompanying pattern (Fig. 1). The

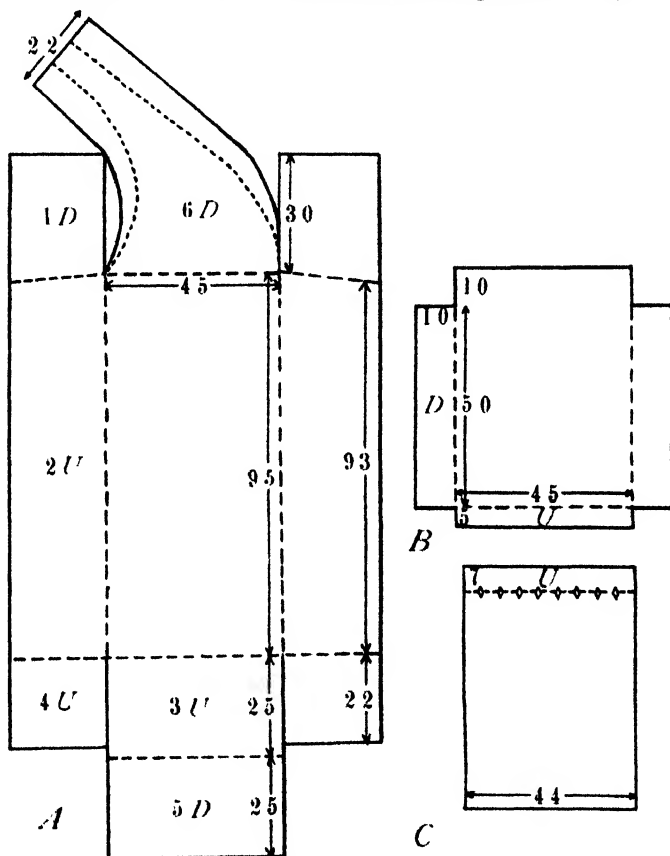


Fig. 1. Pattern of ice carrier

Cut along the continuous lines, then bend along the broken lines in the direction and order indicated. Thus the piece 1 D is first bent down, then 2 U is bent up, etc. The direction and order for the right side is the same as indicated for the left. A, Carrier; B, Cover; C, Straining plate (60 m.m. greatest length). The numbers represent the dimensions in millimeters.

cutting may be done with ordinary scissors. The material is then folded thru 90° along the broken lines, and in the direction and order indicated. The bends are best made against the rectangular edge of a block of wood or similar object. The tubular end of the spout may be formed by molding it around a cylindrical pencil; it is better to curve the back edge of the cover (Fig. 1, B) than to bend it thru a right angle.

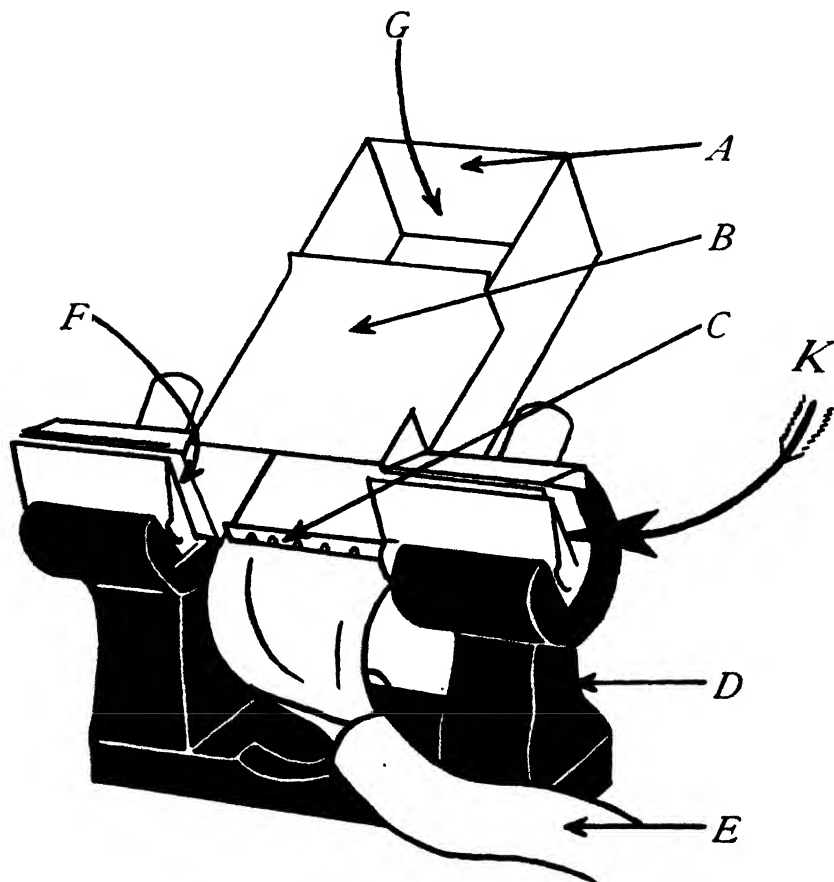


Fig. 2. The assembled ice carrier in position (knife omitted)

A, Carrier; B, Cover; C, Straining plate; D, Knife carriage; E, Drainage tube; F, Wing of ice carrier; G, Ice chamber; K, Insert knife here.

The screws of the knife-clamps are run down against the wings of the device and hold it against the back of the blade. It is then filled with chopped ice, which melts against the blade and flows away thru the tubular spout into a rubber drainage tube. Inclusion of the

perforated straining plate (Fig. 1, C) in the bottom of the device, with the bent edge filling the gap between the base of the blade and the floor of the ice carrier, will prevent slivers of ice from passing under the knife. The completed and assembled ice carrier in position on a Spencer No. 820 rotary microtome knife carriage is shown in Figure 2.

The paraffin block is best mounted on a wooden carrier, chilled, and clamped into the microtome in such a way that it projects considerably beyond the clamp. Generally the block remains sufficiently cooled by its momentary contacts with the knife. It may from time to time be necessary in very hot weather to touch a piece of ice directly to the upper side of the block, gently drying it afterwards. A piece of paper toweling placed on the cover will prevent the ribbon from catching in the moisture which may condense there.

This cooler has proved exceptionally helpful in the cutting of rather thin (3 to 4 μ) sections during the summer or in a warm laboratory. The accompanying design is for an ice carrier adapted to the Spencer No. 820 rotary microtome, and its dimensions may require slight alterations to fit others.

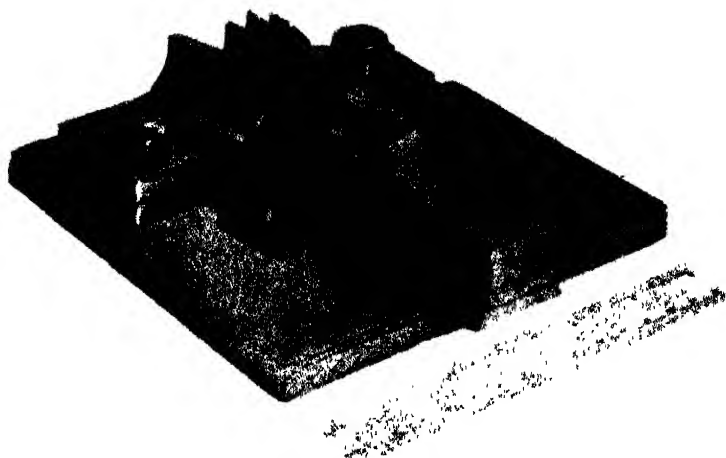
This device was suggested by a method used by Dr. B. R. Speicher.

A SIMPLE TRIMMER FOR PARAFFIN BLOCKS

F. A. WATERMAN, *Department of Zoology, Ohio State University,
Columbus, Ohio*

One of the factors which stands in the way of good sectioning is trimming the paraffin block so that its edges are absolutely parallel. A straight ribbon is always to be desired especially if serial sections are wanted. If the paraffin block is rather large, little difficulty is experienced in trimming it by hand. If the block is small, however, it is often very difficult to get the edges parallel; therefore, most technicians trim their blocks much larger than necessary. It is then impossible to get many sections on a single slide.

The accompanying illustration shows a simple device for trimming paraffin blocks.



Two safety razor blades, of the heavy backless type, are mounted upon two movable metal supports. These blades are held in place by tapered brass plates. These two supports are machined accurately so that the razor blades are perfectly parallel and are keyed into a flat metal base. After the supports have been adjusted to the correct position, they are held in place by thumb screws.

The microtome block carrier is placed in another piece of metal which is mounted upon a brass plate. This brass plate is keyed into the base. It is very important to fit this piece very accurately be-

cause there must be no vibration as the paraffin block passes between the blades.

The block should be cut to approximate dimensions before using the trimmer. If the tissue has been thoroly infiltrated and oriented in the center of the paraffin, the ribbon will be perfectly straight, regardless of its size.

Any machinist can make this trimmer with about eight hours of work. The one pictured above is made of cast iron, altho cast brass or aluminum would probably be better metals to use since they both machine more easily.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

MICROTECHNIC IN GENERAL

CONGER, P. S. The use of hollow ground slides made with the dental engine. *Science*, 83, 5312. 1936.

Additional information on the procedure for making hollow ground slides with a dental engine includes the use of a block for holding the slide. It has the following advantages: it holds the slide securely while drilling; it facilitates centering the concavity on the slide; the lubricant is prevented from running off the slide while drilling; and the slide is protected from excessive scratching in case the drill point should slip. The block is made from hard wood or metal, $\frac{1}{2}$ in. thick and slightly larger than the slide. A depression the size of a slide is cut into one side. A $\frac{3}{8}$ in. hole, countersunk on top, is drilled thru the middle of the block.—*J. A. de Tomasi*.

FABERGÉ, A. C. and LA COUR, L. An electrically heated needle for paraffin embedding. *Science*, 84, 142. 1936.

A heating wire, connected to an electric circuit, is passed thru the eye of a bent sewing needle. This is inserted in the end of a wooden plug with 2 grooves cut along the sides to hold the circuit leads. The heating wire, 4 cm. long, is of small gauge nickel chromium, with a resistance of 60 ohms per meter. Using a transformer with 3.3 volts output, the current will be about 1 ampere.—*J. A. de Tomasi*.

MATTAS, C. L. The adaptability of the Lidberg paraffin embedding oven for various types of tissue work. *J. Lab. & Clin. Med.*, 21, 830-2. 1936.

The Lidberg embedding oven is available in two sizes. The large model can be converted into a drying oven for a large number of slides by fitting it with a rack of six perforated tin shelves. The small model can be outfitted with a rack of 20 shelves for unusually large sections. It can also be provided with 2 receptacles for paraffin, kept ready at constant temperature. These are provided with a spigot and slide on rails. Such modified ovens can be ordered from the manufacturer, Arthur H. Thomas Company, Philadelphia, Pa.—*J. A. de Tomasi*.

ANIMAL MICROTECHNIC

BACSICH, P. On the staining of lipid granules in leucocytes. *J. Anat.*, 70, 267. 1936.

A short, simple, and reliable method for staining lipid granules in leucocytes with Sudan III is given.

Staining Solution: Place 1 g. of finely powdered Sudan III in 96% alcohol, 200 cc. Boil over a water bath for 5 min. in a loosely stoppered liter flask. Add 5% (by vol.) melted phenol. Shake thoroly and filter while still hot into a well stoppered jar. Keep in ice-chest 24 hr. Filter. Add drop by drop enough dist. water to reduce the alcoholic concentration to 80%. Keep at room temp. 24 hr. Filter. Prepare the staining solution freshly from this stock solution by adding drop by drop an equal amount of dist. water. This solution is colloidal and is used without filtering.

Spread a film of blood on a carefully cleaned slide with cover slip (a particularly thin film is obtained by tilting the cover slip at an angle of 25° to the slide). Dry film in air or at 36°C . 30-60 min. Cover film with stain and keep at 56°C . 5 min. Wash with 40% alcohol 1 min. Wash thoroly in running tap water 1-2 min. Counterstain (preferably Weigert's iron hematoxylin) 30-60 sec. Rinse with tap water. Differentiate in acid alcohol 2-3 sec. (HCl , 0.5 cc.; 40% alc., 100 cc.). Wash in tap water a few minutes. Dry in air or at 36°C . Cover with glycerin (cedar-wood oil dissolves Sudan III) and examine immediately. The preparations fade rapidly in air. This can be delayed by keeping in pure glycerin or by mounting with Zwemer's glychrogel.—*H. D. Reed*.

BARBER, M. A. The time required for the examination of thick blood films in malaria studies, and the use of polychromatophilia as an index of anemia. *Amer. J. Hyg.*, 24, 25-31. 1936.

This paper consists of two parts. The first deals with the technic of thick film survey work. Details for making thick and thin films are given. It is suggested that each examiner determine for himself the ratio between the amount of work required and the parasite percentages obtained. In the author's own case the following ratios were found: 150 fields examined, in each case gave 34% positive; 125 fields, 33.4%; 100 fields, 32.7%; 75 fields, 30.9%; 50 fields, 28.5%; and 25 fields, 25.3%. Therefore, the examination of 100 fields was sufficient for survey purposes.

In regard to the relation of polychromatophilia to anemia, the author finds a coefficient of correlation of 0.438 ± 0.022 when hemoglobin percentage is compared with polychromatophilia. It is, therefore, possible to predict macroscopic anemia from polychromatophilia in groups of considerable size, altho the method is not reliable in individuals. In general, there is also a relation between the parasite index and polychromatophilia altho, in individual cases, there may be wide variations from the average.—*George H. Chapman*.

GORDON, H. and SWEETS, H. H., JR. A simple method for the silver impregnation of reticulum. *Amer. J. Path.*, 12, 545-51. 1936.

Fix in 10% aq. formalin or in Bouin's solution. Cut frozen sections or embed blocks in paraffin or celloidin. Affix frozen or paraffin sections to slides by Wright's technic or by Masson's gelatin glue method, or ensheath in celloidin by Warthin's molasses-celloidin sheet method. Oxidize for 1-5 min. in acidified KMnO_4 solution: 0.5% aq. KMnO_4 , 47.5 cc.; and 3% H_2SO_4 , 2.5 cc. Wash in water. Bleach until white in 1% oxalic acid. Wash in tap water and 2 changes of dist. water. Mordant for 15-30 min. (or longer) in 2.5% aq. Fe alum. Wash in 2 or 3 changes of dist. water. Impregnate for a few sec. in diammino silver hydroxide. Wash in dist. water. Reduce in 10% aq. formalin. Wash in water. (If the sections are overimpregnated, repeat the process beginning with mordant.) Tone in 0.2% AuCl_3 1-3 min. Wash in tap water. Fix in 5% $\text{Na}_2\text{S}_2\text{O}_3$ for 5 min. Wash well in tap water. Dehydrate in 80% and in 95% alcohol. For sections fixed by Wright's method, complete dehydration in abs. alcohol and dissolve celloidin in equal parts of abs. alcohol and ether. For celloidin or celloidin sheet sections, complete dehydration in carbol-xylene (xylene 2 parts and phenol 1 part). Clear in xylene. Mount in balsam.—*J. A. Kennedy*.

GRÖAT, W. A. A general purpose polychrome blood stain. *J. Lab. & Clin. Med.*, 21, 978-82. 1936.

It is claimed that this modified Jenner stain is easily prepared and is simple to use. The stain is prepared as follows: *Solution 1*: Eosin Y (C. I. No. 768, 94% dye content) 6 g.; dist. water, 500 cc. *Solution 2*: Methylene blue (C. I. No. 922, 88% dye content) 5 g.; methyl violet 2B (C. I. No. 680, 81% dye content) 1 g.; thionin (C. I. No. 920) 0.2 g.; dist. water, 50 cc. (National Aniline and Chemical Company, Commission Certified dyes were used thruout.) Mix solutions 1 and 2, warm gently to 50°C . and hold 24 hr. at 37°C . Filter thru hard paper, wash residue 3 times with dist. water (last water should be pale blue). Dry thoroly and pulverize. Suspend 0.5 g. powder in 100 cc. methyl alcohol, C. P., stand 2 weeks, shaking frequently. Flood the slide with the stain for 5 min. Plunge, without draining, into 50 cc. of neutral dist. water, mix and let slide stand vertically until it becomes rose pink. Air dry. For staining several slides at one

time: hold 5 min. in a Coplin jar containing the stain; put 5 min. in a second jar containing the stain diluted 1:10; transfer to a third jar containing distilled water and leave until pink. For photomicrographic purposes, where purplish tints are required, the staining time must be prolonged.

Results: Nuclei of polynuclears stain dense blue; nuclei of large lymphocytes, pale purplish blue; small lymphocytes, deep purplish blue; nuclei of monocytes, reticulated grayish blue; nuclei of normoblasts, bluish black; neutrophile granules, bright rose pink; so-called "toxic granules," blue; eosinophile granules, bright pink; basophile granules, purple; the color of the cytoplasm of leucocytes varies as in Wright's stain; malarial parasites, greenish blue with pink to magenta chromatin; blood platelets lavender with magenta chromatin; erythrocytes, bright rose pink.—*J. A. de Tomasi.*

HOWDEN, A. L. III. A rapid and reliable modification of the Weigert-Pal technique suitable for class purposes. *J. Roy. Micro. Soc.*, 56, 29-30. 1936.

The time-consuming Weigert-Pal technic for medullated nerve fibers has been the object of a great many modifications, none of which has been suitable for class work because of the excessive time required. A modification is suggested which can be completed in 48 hr. and is more economical. Fix 24 hr. in formal saline (40% formaldehyde, 10 cc; 1% NaCl in dist. water, 90 cc). Do not wash. Freeze, and cut 20 μ sections. Wash in 1 or 2 changes of dist. water, for a black stain of nerve fibers, mordant in 4% aq. Fe alum. For a blue stain, mordant 1 hr. at 37° C. in 2% aq. (NH₄)₂ MoO₄. Wash in 2 or 3 changes of dist. water. Stain 1 hr. at 37° C. in Kultschitzky acid hematoxylin. Pass thru dist. water, and for 30-60 sec. in 0.25% aq. KMnO₄. Transfer and agitate for 30-60 sec. in the following solution: Sat. aq. oxalic acid, 4 cc., sat. aq. Na₂S, 4 cc., dist. water 92 cc. Wash quickly and repeat treatment in KMnO₄ and latter solution until the desired differentiation is obtained. Wash twice in dist. water and leave in tap water 20 min. Counterstain with erythrosin, eosin, neutral red, or Van Gieson's combination. Mount on gelatinized slides (Müller, E. J. Frozen section technique. *J. Roy. Micro. Soc.*, 50, 302. 1930) or pass quickly thru methylated spirit and abs. alcohol. Clear and mount in balsam.—*J. A. de Tomasi.*

IKEDA, S. Über die elektrostatische Ladung des Augapfels. III. Mitteilung. Zusammenhang zwischen den IEP und einigen mikrochemischen Reaktionen beim Augapfel des Herbstfrosches. *Folia Anat. Jap.*, 14, 175-80. 1936.

In this study Unna's methyl green manganese method (Unna, P. G., und Luise Fezer. *Zur Färbung der Nervenfasern an frischem Gewebe. Virchow's Archiv.*, 246. 1923) and Feulgen's stain were used in studying the isoelectric point of cells of the eye pupil in the frog. The object was to determine the relationship of the isoelectric point to the oxidation-reduction potential of the cells, especially with reference to staining capacity of the nucleus. Results show that isoelectric point and oxidation-reduction potential are directly related, i. e., those nuclei and plasmas that display a lower isoelectric point are the seat of stronger oxidation reactions. No connection was found between isoelectric point and reducing power or strength of the Feulgen nucleal reaction.—*J. A. de Tomasi.*

KNEBERG, M. Differential staining of thick sections of tissues. *Science*, 83, 561-2. 1936.

Thin sections of human scalp do not give a clear idea of the relationship of the hair follicle to its appendages and adjacent tissues. The following technic applies to sections from 100 to 400 μ thick, suitable for high and low-power study. Embed in low viscosity nitrocellulose. Cut and remove nitrocellulose as follows: Abs. alcohol, 1 hr.; equal parts ether and abs. alcohol 24-36 hr.; change solution once; 75% alcohol, about 30 min.; dist. water, 30 min.; (for tissues fixed in Zenker's solution: Lugol's solution, 12-24 hr.; 5% Na₂S₂O₃ solution until white, 1-2 hr.; dist. water, 8-12 hr.).

Stain in hematoxylin-eosin as follows: Delafield's hematoxylin, 5-8 drops in 50 cc. dist. water, 8-12 hr.; tap or dist. water containing a few drops of Li₂CO₃ until nuclei are blue, 5 hr.; eosin solution, pale pink in dist. water, until sections

are pale pink; abs. alcohol, 2 changes, 3-5 hr.; xylol until clear, 1-2 hr.; mount with dammar above and below the section.

Two further staining procedures are also suggested, one using hematoxylin-eosin-azure II, the other Fe hematoxylin.

If the removal of the nitrocellulose is preferred following the staining, the procedure is: 75% alcohol, 1 hr.; 95% alcohol, 3-5 hr.; creosote solution (1 part creosote, 1 part toluene, 2 parts xylene) until completely clear. Mount in dammar.—*J. A. de Tomasi.*

LEPINE, P. and SAUTTER, V. *Méthode de fixation histologique rapide et colorations cytologiques du nevraxe.* *Bull. d'Histol. Appl.*, 13, 287-9. 1936.

For a rapid diagnosis of rabies, also applicable to the central nervous system, a new fixative is recommended which has the advantage of more rapid and better fixation and staining. Mix equal volumes of: glacial acetic acid, acetone, and sat. alc. (abs.) HgCl_2 . Before staining remove the HgCl_2 from sections by passing thru Lugol's solution or iodine alcohol. Brain tissue fragments of 1 mm. thickness will fix in 15 min. Pass thru 2 baths of abs. alcohol to dehydrate; 15 min. in 2 baths of xylene; 15 min. in 2 changes of paraffin. A whole rabbit brain requires 6-8 hr., guinea pig brain, 5 hr. For large pieces, it is advisable to change the fixative at least once.—*J. A. de Tomasi.*

MORRISON, S., GARDNER, R. E. and REEVES, D. L. *The selective elimination of neutral red through the gastric mucosa.* *J. Lab. & Clin. Med.*, 21, 822-7. 1936.

Experimental work with laboratory animals proves most successful on starved white rats. Shortly after intravenous injection of 1 cc. of 1% aq. neutral red, whole stomachs are fixed (no time given) in the "Susa" mixture of Heidenhain: HgCl_2 (4.5 g.), NaCl (0.5 g.), dist. water (80 cc.); before use add: trichloroacetic acid (2.0 g.), acetic acid (4 cc.), formol, (20 cc.). Transfer to 90% alcohol and on same day pass thru abs. alcohol. Cut stomach longitudinally. Pass into CS_2 ; soft paraffin and CS_2 , 1:1. Soak 8-12 hr. in soft paraffin. embed in hard paraffin. Cut, carry thru alcohols, clear and mount in neutral balsam. Neutral red is retained in the canaliculi of the parietal cells. Localization of the granules of dye corresponds exactly to the anatomic distribution of parietal cells stained with phloxine-methylene blue. As neutral red is selectively eliminated, it may be used as a test for true achylia and might help in interpreting the mechanism of pernicious anemia.—*J. A. de Tomasi.*

MUNRO, S. S. *Preparation of avian sperm smears for microscopy.* *Science*, 83, 532. 1936.

The elongated heads of avian sperm offer technical difficulties in the preparation of dry smears. They often become contorted and twisted, assuming a spiral or corkscrew shape. To obviate this, two procedures are recommended.

Mounted Preparations: Dilute semen with 2-4 volumes of Ringer's solution. Smear coverslip lightly with albumen fixative and spread a drop of the suspension on it. Drain off any excess with blotting paper, fix by suspending 30-60 min. in the vapors above a 2% osmic acid solution contained in a small airtight jar. Wash repeatedly in water, mordant 30 min. in ferric alum and stain for 1 hr. or longer in Heidenhain's hematoxylin. Wash out the stain from the acrosome, middle piece and tail by immersing for a few seconds in ferric alum (time must be carefully controlled). Run up to 95% alcohol and counterstain with 1% light green (SF yellowish?). Rinse in 95% alcohol, dehydrate in abs. alcohol, clear in xylene, alcohol and xylene. Mount in balsam. Preparations with well-stained tails are difficult to obtain. Osmic acid, as a fixative, is preferable to 15-20% formalin.

Dry Smears: Dilute semen with normal saline, place a drop near one end of slide and quickly draw out to a thin film with a slip of paper. The film must be thin enough to dry instantaneously. Fix overnight in an oven at 100° C. Stain 30-60 min. in fresh Ziehl-Neelsen's carbol fuchsin. Wash and counterstain up to 1 min. with aq. methylene blue (strength not stated) at 40° C. Such dry smears may be preserved indefinitely in balsam mounts.—*J. A. de Tomasi.*

NISHIMURA, T. *Histologische Studien über die Antiserumwirkung. II. Färbbarkeit der Erythrozyten bei Zusatz von Normal und Antiserum in vitro.* *Folia Anat. Jap.*, 14, 1-14. 1936.

This is a study of the physico-chemical properties of fresh and fixed erythrocytes in the presence of normal and antiserum, by using saline solutions of the following dyes obtained from G. Grüber and Co.: acid dyes—Bordeau R, eosin blue, indigo carmine, trypan blue; basic dyes—Janus green, Nile blue sulfate, safranin, toluidine blue. A series of rabbits was divided into 3 groups, one used as control, the second injected with a fresh saline suspension of rat erythrocytes, the third injected with a saline suspension of the same red cells fixed in alcohol and ground in a mortar. After a month the blood was collected and the various sera separated and tested with the erythrocytes. The intensity of staining was measured by the concentration of the dye left over in the staining solution, as determined by a Duboscq colorimeter. It was also measured by observing the erythrocytes under a comparison microscope having 2 parallel tubes. Results: fresh cells stained more strongly with basic than with acid dyes; with fixed cells this was reversed. The addition of sera tended to nullify differences in the intensity of staining capacity between acid and basic dyes. Fresh erythrocytes showed greater capacity for taking up stain with increasing concentrations of sera; fixed cells stained better with basic dyes than with acid dyes. Fresh erythrocytes were stained much more strongly in the presence of normal serum than with the antiserum. Their negative electric charge was also lowered by the antiserum.—*J. A. de Tomasi.*

RASMUSSEN, A. T. *Copper hematoxylin, a stain for the acidophils of the human hypophysis.* *Proc. Soc. Exp. Biol. and Med.*, 34, 760-2. 1936.

Sections fixed in formalin, Bouin's, Helly's, Regaud's, Zenker's and Zenker-formol (half the acetic acid replaced by formalin) fluids gave essentially the same results. Acidophils of the human hypophysis, but not basophils, were fully differentiated by the copper hematoxylin method (given in McCullung's Microscopical Technic, p. 203, 1929).—*M. S. Marshall.*

SCHARADASCH, A. *Histophysiologie des réactions réciproques entre le bleu de méthylène et le tissu nerveux. I. Influence de l'équilibre d'oxygène.* *Bull. d'Histol. Appl.*, 13, 5-27. 1936.

This is a study of nerve tissue elements by means of intravital staining. The progress of staining is controlled by observation of the specimen *in toto*, it being unnecessary to dissect or make sections. Unlike standard histological methods, this technic depends upon the transparency of living tissues. Staining is accomplished by injecting physiological solutions of methylene blue. Conclusions: Staining is the result of a chemical reaction taking place between nerve tissues and the dye, a process which is comparable to the action of an H^+ acceptor. This reaction of methylene blue with nervous tissue changes our conception of the theory of vital staining.—*J. A. de Tomasi.*

SCHREK, R. *A method for counting the viable cells in normal and in malignant cell suspensions.* *Amer. J. Cancer*, 28, 389-92. 1936.

Resistance of viable cells to staining affords an opportunity of differentiating them from non-viable cells. The following method was used in preliminary studies of the effect of various agents on tumor cells. Grind tissue in a Latapie apparatus and filter the suspension thru 80-mesh Monel metal wire cloth. To 0.2 cc. of suspension add 4.8 cc. of 1:2000 eosin in Tyrode's solution at pH 7.6. Shake 2 min., transfer a drop to a hemocytometer. Stained cells are diffusely pink. Differentiation and counting of viable cells is easily done. Methylene blue does not give as good differentiation. Each dye solution must be tested for its capacity to stain all types of cells promptly in killed suspensions, and to differentiate them in fresh suspensions.—*J. A. de Tomasi.*

STRUMIA, M. M. *A rapid universal blood stain.* *J. Lab. & Clin. Med.*, 21, 930-4. 1936.

A new blood stain is recommended, combining in one solution the Giemsa and May-Grünwald formulas, and is intended to eliminate the deficiencies of either.

Another purpose of the study was to standardize the procedure so as to obtain consistent results with all smears. The stain is prepared as follows: Suspend 1.3 g. of finely ground azure-II-eosin mixture (Giemsa) in 80 cc. glycerin; allow to dissolve and shake occasionally for 2-3 days. Heat and stir 2 hr. in a water bath at 60° C. Cool and dissolve in 290 cc. methyl alcohol and 290 cc. acetone C. P. Prepare separately: 0.15 g. methylene-blue-eosin powder (May-Grünwald), 170 cc. methyl alcohol, 170 cc. acetone, C. P. Shake occasionally during several days until dissolved. Mix the two solutions. *Staining*: Flood fresh smears for 2 min. with 1 cc. staining solution, keeping slides covered with dish or bell-jar. Add 1 cc. alkaline water (0.2 cc. of 1% aq. Na_2CO_3 and 100 cc. dist. water); stir, and allow to stand for 3 min. Wash in running water. Air dry. The 1% Na_2CO_3 solution should be renewed every 2 months, the diluted alkaline solution every 2 or 3 days. It is better to allow the fresh staining solution to stand for 2 weeks before determining if any alkali is necessary. If the stain is too acid, the smears appear bright red (overstaining of erythrocytes and eosinophiles); if too basic, the smears appear bluish. A proper reaction of the alkaline diluent causes an iridescent sheen to appear over the stain. Solutions two or more years old are not dependable. Such a stain is now prepared by the Coleman and Bell Co.—*J. A. de Tomasi*.

YASUZUMI, G. and MATSUMOTO, S. Über den isoelektrischen Punkt der tierischen Gewebe. VI. Mitteilung. Nochmals IEP der Erythrozyten einiger Tiere. *Folia Anat. Jap.*, 14, 101-6. 1936.

This is the 6th contribution of a series of papers on the isoelectric point of animal tissues. A method for determining the isoelectric point of mammalian erythrocytes is reported. Red cells from man, rabbit, cat and guinea pig were used. After separation from serum, the cells, in 10% suspension, were fixed with abs. alcohol, washed repeatedly with dist. water and stained with 0.01% toluidine blue or 0.005% ponceau at varying H-ion concentrations. After 30 min., and again after 3 hr., the erythrocytes were removed by centrifuging and their dye content determined colorimetrically with the rovinbond tintometer. The technic used was adding 1 cc. of the centrifuged cells to 10 cc. of 2.5% acetic acid and 1 cc. H_2O_2 (perhydrol, Merck) and heating 5 min. in a boiling water bath.

The isoelectric point of the blood cells was shown to lie on the acid side. This is in agreement with previous measurements also obtained by the same author and reported elsewhere.—*J. A. de Tomasi*.

PLANT MICROTECHNIC

HEITZ, E. Die Nukleal-Quetschmethode. *Ber. deut. botan. Ges.*, 53, 870-8. 1936.

Two methods for making root tip smears for cytological study are described (1) the heat method (2) the nucleal reaction (Feulgen reaction). Using the first method the result may be secured in two ways depending upon the type of material. The root tips are placed in aceto-carmine, and boiled vigorously. The material thus treated is cut into thin strips; placed on a slide, and flattened by pressure on the cover glass. An alternative procedure is to section the material by hand. These sections are placed on a slide in a drop of aceto-carmine, and brought to a boil. In properly made preparations, slight pressure on the cover glass is sufficient to spread the material for observation. In the second method the material is fixed in chromium-osmic acid, 15-30 min.; hydrolyzed in hot 50°-60° N HCl, 15-30 min., then placed on a slide in 45% acetic acid. The material is cut into fine pieces and the cover added. The latter method is recommended for investigation of the finer morphological details of somatic chromosomes that would be obscured by clumping if the heat method were used.—*Thomas W. Whitaker*.

NEWBY, W. W. and PLUMMER, P. Technique for preparing microscopic sections of woody stems and roots. *Bot. Gaz.*, 98, 198-9. 1936.

The following method is similar to that used in the preparation of slides of petrified wood: Cut material into sections 2-4 mm. thick, having parallel surfaces. Smooth one face of the section by rubbing it on coarse sandpaper attached to a glass plate. This removes the saw marks. Finish with finer sandpaper and

polish with the finest grade of emery. Mount on a glass slide with Canada balsam. Dress down to desired thickness with fine sandpaper and emery paper. Finish with clean worn emery paper by applying very little pressure on strokes. Remove and wash with xylene and remount in balsam. Sections can be made as thin as 10 μ , requiring from 30 min. to 4 hr.

Before finishing the section it may be stained (for a few hours or up to 1 day, according to the hardness of the wood) in solutions of certain dyes. For this purpose iodine green, with orange G as counterstain is often satisfactory; but the authors secured best results with a solution of 2 g. acid fuchsin in 100 cc. sat. alc. picric acid. A 2% aq. AgNO_3 solution applied for 2-3 min., followed by a wash with dist. water was found to develop a brown differential stain upon exposure to strong light. Staining, however, is not essential for obtaining most satisfactory slides.—*J. A. de Tomasi.*

MICROÖRGANISMS

DU, S. D. Simple and rapid methods of staining *Treponema pallida*. *Chinese Med. J.*, 50, 1283-5. 1936.

Preparation of Smears: A loopful of the serum from the lesion is spread on a clean glass slide. When examining an enlarged syphilitic gland, 0.5 cc. of sterile saline is injected into the gland and then aspirated with a 1 cc. syringe using a 22-gauge needle. These smears are dried in the air. If necessary, dehemoglobinize with distilled water.

Modified Gentian Violet: Four grams of powdered gentian violet (methyl violet?) are dissolved in 4 cc. of abs. alcohol by grinding in a mortar with the gradual addition of 50 cc. of doubly filtered anilin water. The anilin water is prepared by mixing 4 cc. of anilin oil with 96 cc. of dist. water. Filter twice thru filter paper.

Method A: Stain for 2 min. with the following: Modified gentian violet, 8 drops; add immediately (to minimize and remove precipitate) 5% alcohol, 8 drops; follow immediately with alcoholic NaOH (abs. alcohol, 3 cc. plus 1% NaOH, 100 cc.), 8 drops. Wash in running tap water for 20 sec. and dry.

Method B: Stain for 2 min. with the following: Modified gentian violet, 8 drops; add immediately 5% alcohol, 8 drops; follow immediately with 5% K_2CO_3 , 8 drops. Wash in running tap water for 10 sec. and dry.

Method C: Stain for 2-5 min. in modified gentian violet, 8 drops; follow with 1% NH_4OH , 8 drops; immediately add 5% alcohol, 8 drops. Wash in running tap water for 10 sec. and dry.

Method D: Stain as follows: Modified gentian violet, 8 drops; immediately follow with 1% NH_4OH , 8 drops; 1% KCl, 8 drops.

With any of these combinations, *Treponema pallida* stains intensely purple. The regular spirals are distinct and free from precipitate. Other spirochaetes, such as *Spirochaeta refingens*, stain purplish black with irregular open and coarse spirals.—*J. A. Kennedy.*

MAC NABB, A. L. Cultural methods of isolation of tubercle bacilli. *Amer. J. Pub. Health*, 26, 619-24. 1936.

These are procedures used in the Ontario Department of Health Laboratories, with a study of several culture media. The most satisfactory method of handling specimens preparatory to culture, is to treat them with equal parts of 3% HCl for 2 hours, followed by neutralization with 3% NaOH. Brom cresol indicator (brom cresol purple?) is added directly to the specimen-acid mixture before treatment. Of the media used, Lowenstein's and Petragnani's yielded the greatest number of positives. Woolley's medium showed a rather high percentage of contaminated tubes, but when malachite green was added to it instead of crystal violet, very satisfactory results were obtained.—*M. W. Jennison.*

POWELL, W. N. *Trichomonas vaginalis* Donné 1836: Its morphologic characteristics, mitosis and specific identity. *Amer. J. Hyg.*, 24, 145-69. 1936.

This is an excellent, well illustrated discussion of the cytology of this protozoan and a comparison of it with closely related trichomonads. The author concludes that *Trichomonas vaginalis* differs from buccal and intestinal trichomonads of man and is a distinct species.—*George H. Chapman.*

STONE, W. S. A method of staining protozoa in bulk. *J. Lab. & Clin. Med.*, 21, 889-42. 1936.

This technic satisfies the following requirements: concentration of organisms, fixation and staining in bulk, no distortion of internal structure of organisms, and proper differentiation. *Concentration and fixation:* With fecal specimens, emulsify 20 cc. in 200 cc. of saline at 37° C. in a tall cylinder, let it settle for 5 min. and decant supernatant liquid into two 50 cc. centrifuge tubes. Centrifuge 5 min. at 1850 r. p. m.; decant the supernatant liquid and test the sediment of one tube. Fix the second with Schaudinn's solution for 1-2 hr. For other liquid specimens, pipe the fluid into a 50 cc. centrifuge tube and proceed as above. *Staining:* (All following steps performed by centrifuging.) Wash fixed material twice with dist. water. Wash 10 min. in 70% alcohol with enough Gram's iodine added to make it light brown. Wash 10 min. with 70% alcohol. Stain 1-24 hr. with Harris' or Delafield's hematoxylin. Wash in tap water. Decolorize with 20 cc. acid alcohol (1% of HCl in 70% alcohol) until, under the high dry power of microscope, cytoplasm appears practically colorless. Add sufficient ammonia water (5 drops NH_4OH to 50 cc. dist. water) to neutralize the acid and turn the solution bright blue. Wash with tap water, dehydrate 10 min. each with 70%, 95%, 95%, abs. alcohol and abs. alcohol. Clear in xylene. Suspend in xylene, add sufficient Canada balsam to make a syrupy mixture, and mount.—*J. A. de Tomasi.*

WALD, H. and VAN WINKLE, C. C. A Comparison of the Ziehl-Neelsen and Spengler technics of staining the tubercle bacillus. *J. Lab. & Clin. Med.*, 21, 844-7. 1936.

For this study only positive sputum cases with only few organisms per oil immersion field were utilized. Sputum in Groups I and II of the Gaffky scale, with a few from Group III are selected for study. The 2-slide method gives reliable and even smears; these are heat fixed and placed in cold carbol fuchsin over night. For Ziehl-Neelsen method, decolorize with acid alcohol and counterstain lightly with Löffler's methylene blue; for Spengler's method, dip for 5-10 sec. in picric acid alcohol (1:1 sat. aq. picric acid and 95% alcohol), wash in 60% alcohol, decolorize 10-30 sec. in 15% aq. HNO_3 and counterstain 30-60 sec. in picric acid alcohol. Statistical analysis of results discloses that there is a slight, but fairly consistent, advantage in the Spengler method. Improvement, however, is not high enough to warrant a change in the laboratory routine.—*J. A. de Tomasi.*

HISTOCHEMISTRY

CASTEL, P. Recherches sur la détection histochimique du bismuth. *Bull. d'Histol. Appl.*, 13, 290-97. 1936.

Two technics are suggested for the detection of Bi in animal tissues. *Technic 1:* Fixation is not connected with reactions for the metal. Fix in 10% formol, wash 12 hr. in running water, dehydrate and embed in paraffin. Run down to water. Treat 15 min. in the following solution: dissolve 1 g. brucine in 100 g. dist. water by the addition of 3-4 drops of concentrated H_2SO_4 , warm slightly and add 2 g. KI. Wash shortly in dist. water, mount in Apathy's syrup. A nuclear stain with methyl violet may follow the brucine reagent. The granules of Bi appear bright red and the nuclei purple. *Technic 2:* Fixation and detection of the metal are done simultaneously. The reagent used is made up of alcohol and chloroform 1:1, 100 cc.; KI, 2 g.; brucine, 1 g. Further details of technic not given.—*J. A. de Tomasi.*

CASTEL, P. Recherches sur la détection histochimique de l'arsenic. *Bull. d'Histol. Appl.*, 13, 106-12. 1936.

Two technics are suggested for the histochemical detection of As in adult guinea pig tissues. Na_2AsO_4 was injected in the form of a 1% aq. solution, also as Pearson's liquid using a total of 0.5 g. Na_2AsO_4 .

Technic No. 1: Fix for 5 days in the following solution: 10% formol, 100 cc.; CuSO_4 , 2.5 g. Wash 24 hr. in running water. Embed in paraffin. Cut and stain lightly with eosin for a background color. The green particles of hydro-copper arsenate are made visible within the cells. In the above formula the same amount of copper acetate can be substituted for the sulfate.

Technic No. 2: Fix for 12-24 hr. in the following solution: abs. alcohol, 50 cc.; chloroform, 50 cc.; conc. HCl, 3 cc. Saturate with H_2S . Material thus treated can be stained lightly with hematein-eosin. As appears in the form of yellow granules within the cellular elements.—*J. A. de Tomasi.*

FEYEL, P. *La recherche histochimique des chlorures dans les cellules rénales.* *Bull. d'Histol. Appl.*, 13, 289-307 1936.

This paper is a discussion of the method devised by Leschke for the determination of chlorides in animal tissues. The method is based on the insolubility of AgCl in acid solutions. Small tissue fragments (0.5-1.0 mm.) are fixed 24 hr. in the dark with a mixture 1:1 of 2% $AgNO_3$ and 2% HNO_3 . After washing several hours, the Ag is fixed with Cajal's solution, which acts also as a reducing agent. Any AgCl is reduced to black granules. The paper is largely an answer to criticisms raised by Lison regarding the localization, specificity, and other conditions of the reaction.—*J. A. de Tomasi.*

MARZA, V. D. *Contribution a la méthode de Macallum pour la détection histochimique du potassium.* *Bull. d'Histol. Appl.*, 13, 62-71. 1936.

A modification is reported of the McAllum technic for the detection of K in tissues. Reagents: dissolve 6.5 g. of $Na_2Co(NO_2)_6$ in 17.5 ml. of dist. water, add 5 drops of glacial acetic acid. Prepare also a 3-5% aq. solution of $(NH_4)_2S$. Fix material in 96% alcohol in the icebox, embed in paraffin and cut.

As a blank test, treat sections for 5 min. in a closed jar with the $(NH_4)_2S$ solution, wash thoroly with dist. water, run up thru the alcohols, xylene, and mount. For the detection of K, cover the sections for 2 hr. with the Co solution. Dip slowly and keep for 30 min. in 50% alcohol. Wash in 3 changes of 50% alcohol. Cover for 5 min. with the $(NH_4)_2S$ solution. Wash thoroly with dist. water. Finish as with the blank.

Conclusions: The Co reagent is precipitated by inorganic as well as organic ammonium salts, but is not precipitated by amino acids, urea, or uric acid. Inorganic salts of Fe are precipitated by $(NH_4)_2S$. For this reason the blank test is introduced in order to distinguish Fe from K reactions.—*J. A. de Tomasi.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the October number of this Journal.

STAINS CERTIFIED SEPT. 1, 1936 TO NOV. 30, 1936*

Name of dye	Certification No. of batch	Dye Content	Object of tests made by commission†	Date approved
Light green	NL 1	89%	As histological and cyto- logical counterstain	Sept. 9, 1936
SF yellowish				
Safranin O	CS 7	89%	As bacteriological stain only	Sept. 14, 1936
Safranin O	NS 10	94%	As histological, cytological and bacteriological stain	Oct. 7, 1936
Jenner's stain	NJr 3	—	As blood stain	Oct. 7, 1936
Wright's stain	NWr 10	—	As blood stain	Nov. 13, 1936
Orange G	CO 5	78%	As histological counterstain	Nov. 16, 1936
Eosin Y	CE 8	89%	As histological counterstain and as constituent of blood stains	Nov. 16, 1936
Methyl violet 2B	CMv 3	83%	As histological and bacte- riological stain	Nov. 16, 1936
Carmine	CCa 4	—	As histological and cyto- logical stain	Nov. 27, 1936

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

STAIN TECHNOLOGY

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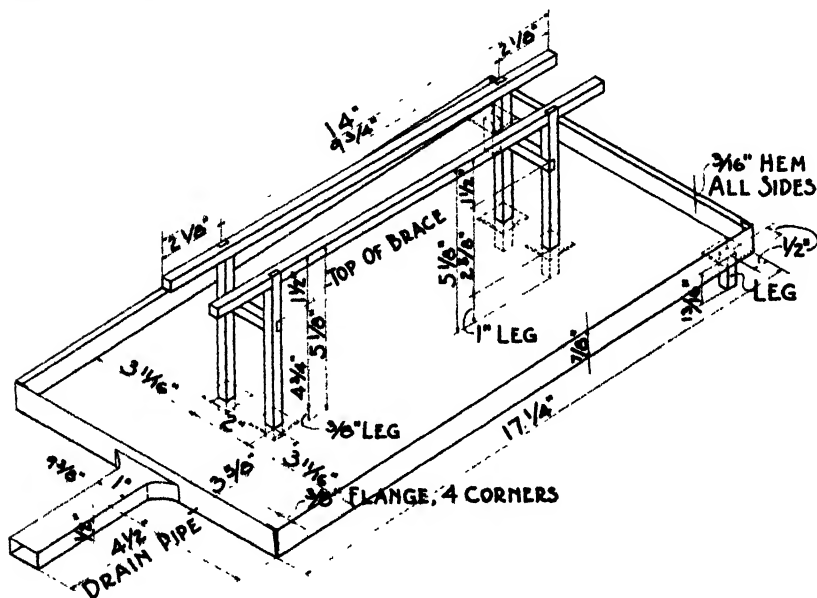
APRIL, 1937

NUMBER 2

A PRACTICAL STAINING RACK

WILLIAM REINER-DEUTSCH, *Doctors Hospital, New York, N. Y.*

The staining rack shown in the accompanying sketch was devised by the writer and has been in use for several years without being considered worthy of public description. Frequent recent comments about it, however, have finally convinced the writer that it may be useful to others.



STAINING RACK

The sketch is very largely self-explanatory; but a few additional matters of detail are needed, e.g.:

1. The staining rack is of $\frac{5}{16}$ " brass bars.
2. All bar joints are to be notched, lapped and brazed.
3. Four of the six legs are to be continuations of the vertical bar-struts of the rack.

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4. All the legs shall be secured to the basin with built-up solder reinforcing around legs on underside of basin.
5. Basin to be of 16 oz. cold rolled sheet copper.
6. All basin corners to be brazed.
7. Drain pipe to be seamless copper tube.
8. Pipe to be brazed to basin.
9. Drain pipe to have same slope as basin, e.g., about $\frac{3}{4}$ " to the running foot.

The advantages of such a rack are rather self-evident, but may be emphasized as follows: One may stain 12 slides at a time without any difficulty. Rubber tubing connected to a faucet is used for washing. The slant of the staining basin offers a quick outflow of stain and water.

Since the above note was written, a staining rack embracing all the features here described has become available commercially and is announced in the advertising pages of this issue.

A TECHNIC FOR THE SECTIONING OF MAMMALIAN OVA AND BLASTOCYSTS¹

W. W. GREEN,² CATHERINE BARRETT, and L. M. WINTERS,
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St. Paul, Minnesota*

ABSTRACT.—Ova and blastocysts are fixed in Bouin's fluid. Gradual dehydration thru the alcohols to 70% follows. Eggs and blastocysts are stored in 70% alcohol.

The egg.—The 70% alcohol is reduced gradually to 30%. Agar (1.7 g.) is dispersed in 98 cc. distilled H₂O by heat. Hot agar (5–6 drops) is placed on a 50 x 75 mm. microscope slide. After the agar has cooled slightly, the egg is placed in the agar and the agar allowed to gel. The agar is trimmed to about a 2–3 mm. block. The agar with the egg is pushed on a piece of lens paper which is folded about it. The folded lens paper with agar is placed in Bouin's fluid, and the following day run up to 70% alcohol. It is then run back to water and stained with Heidenhain's iron hematoxylin. After being passed thru the alcohols, it is cleared in cedar oil. Embedding in paraffin follows.

The blastocyst.—The blastocyst is stained with Delafield's hematoxylin. It is carried to absolute alcohol. The alcohol is reduced to a minimum and the bottom of the dish coated with glycerin. Cedar oil is substituted for the alcohol. The cedar oil is washed out with a 2–3 minute change of xylol which is replaced with paraffin-xylol. Three changes of paraffin-xylol are made during the next 2–3 hours. Five changes of paraffin are made during 1½ hours of infiltration. All changes of fluid are made under the microscope and by drawing the fluid off and adding new fluid with pipets rather than moving the blastocyst. Between changes of paraffin the bulbs are removed and the pipets placed in a warm oven.

The small size of the mammalian ovum and blastocyst has necessitated the development of special technics for their embedding and sectioning. Hill (1910) and Hill and Tribe (1924) working with the cat fastened the egg to a piece of tissue, brain cortex or fetal membrane, by means of a dilute solution of photoxylin (1–2%). A rather

¹Published as paper No. 1448 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. Contribution from the Animal Genetics Section, Division of Animal Husbandry.

²Formerly assistant and graduate student in the University of Minnesota, now instructor in the University of Alaska, College, Alaska.

elaborate but effective method of handling and infiltrating eggs has been described by Heuser and Streeter (1929) and also by Gregory (1930). By this method ova are transferred from dish to dish by means of fine pipets. At the time of infiltration electrically heated pipets and plates are used. A complete set of equipment as described by Gregory was made and used satisfactorily in this laboratory.

Mammalian ova and blastocysts are fragile and every time either is handled there is a chance of injury. If, however, the egg can be placed in some suitable mechanical carrier which will not damage it, the egg may be moved as quickly and easily as larger pieces of tissue. The finding of a suitable carrier for the egg was reduced to the finding of a gel which could be kept in a sol form at a low enough temperature to permit its use without causing heat injury to the egg and still be of practical use. Agar as used for bacteriological work was found to be satisfactory. It is easy to handle; it remains liquid at a comparatively low temperature; and it does not injure during subsequent treatments.

All the ova and blastocysts recovered for this study were fixed in Bouin's fluid and dehydrated gradually thru the series of alcohols to 70%, remaining in each 30 to 45 minutes. In changing the alcohols, one-third of the original fluid was drawn off and substituted by a larger volume of the next alcohol. Then two-thirds of the alcohol was removed and replaced with fresh alcohol; this was repeated several times. The standard embryological dish was used and the changes were made under a low power microscope.

THE EGG

The 70% alcohol in which the egg has been stored is reduced gradually to a 30% solution. Clean pure agar (1.7 g.) is added to 98 cc. of distilled water and dispersed by heating almost to the boiling point in a covered beaker. The egg is drawn into a fine pipet. (This is the only time the egg has to be moved.) A 50 x 75 mm. microscope slide is placed under the microscope, and 5-6 drops of hot agar placed on the slide. When the agar has cooled sufficiently to prevent heat injury to the egg, the egg is placed in the agar, and the agar permitted to gel. A little distilled water is dropped on the agar; this acts only as a lubricant, and by means of a sharp blade the excess agar is cut from the egg. Each step is carried out under a low power microscope. The remaining agar, about 2-3 mm. square, with the egg in the center is pushed gently from the wet slide onto a piece of lens paper which is then folded about the agar as described

by Guyer (1930). The folded lens paper is placed in Bouin's fluid and the following day run up to 70% alcohol.

Two-celled eggs and occasionally the more advanced stages require orientation. In this case a larger amount of agar is placed on the slide; when it is cool the egg is added and, by means of a hair fixed in a hemostat, the egg may be oriented. If the egg is not oriented properly in the agar, it may be reoriented without harm. As much agar as possible is trimmed from the egg, and the egg with the remaining agar is re-embedded in another agar sol. After the agar has congealed sufficiently, it is cut into an oblong block 3 x 10 mm., the egg being a short distance from one end. The long side of the block should be parallel to the division line between the cells. The end not containing the egg may be cut any shape to differentiate the ends. The agar is later oriented in the paraffin block.

The agar wrapped in the lens paper is transferred easily from dish to dish with the aid of small forceps. The very gradual changes of alcohols as suggested previously for the unprotected egg are unnecessary, because the agar retards contact of the new solution with the egg altho it is very efficient for the exchange of the liquids. (For a discussion of gel structure and formation see Gortner, 1929.)

The agar in the lens paper is run from the 70% alcohol back to water from which it is placed in ferric alum for four hours. It is then stained overnight in Heidenhain's iron hematoxylin. The agar is then passed thru the alcohols (30, 50, 70, 80, 95%, and absolute) leaving it 45 minutes in each. At this time the agar is black. The agar is "cleared" in cedar oil, and allowed to remain in cedar oil at least one hour after it sinks (overnight is a convenient time for this). It is washed with xylol 2-3 minutes and then placed in paraffin-xylol for three hours. Infiltration is accomplished by changes of paraffin (56° C.) every 15 minutes for 1-1½ hours or until the cedar oil is all removed. The warm lens paper is now unfolded. The small block of agar, which is observed easily, is transferred into the embedding paraffin (56° C.) by means of a warm forceps.

No marker is necessary because the black color of the agar indicates clearly its position in the block. The egg is then sectioned at 3-4 μ and placed on slides. At this point the previous staining and the agar are of much benefit; the agar and egg are observed with ease in the paraffin ribbon. A few wrinkles may appear in the agar, but they are removed readily by careful manipulation of very fine dissecting needles, the operation being watched thru the microscope. By this means only the slides containing the sectioned egg need be saved and no time is lost hunting for the egg. After the slide has dried it

is put thru xylol, the alcohols, and water, at which point it is destained carefully with ferric alum. The usual methods of technic are followed from this point to cover-slipping. A counterstain may be given if desired.

The egg stands out clearly and distinctly in the agar which is destained completely.

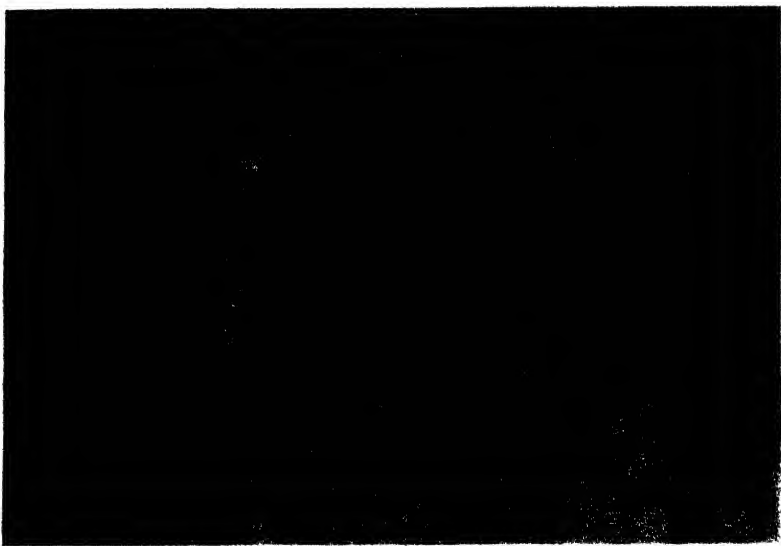


Fig. 1. A rat ovum, recovered from the follicle, sectioned by the method described herein. $\times 740$.

THE BLASTOCYST

For obvious reasons agar could not be used for the blastocysts; they are very fragile and will fragment upon the slightest provocation. Transferring thru the alcohols and other fluids is far more hazardous than the same operation with eggs. A method to reduce handling to a minimum has been developed. Only one transfer is necessary and that cannot be eliminated.

After the blastocyst is found in the flushing fluid, it is transferred to a clean embryological dish containing Bouin's fluid. It is allowed to fix for 12-24 hours after which it is dehydrated gradually to 70% alcohol. It is then stained with Delafield's hematoxylin diluted to one-third strength with distilled water. Following Gregory's suggestion, a very small amount of HCl should be added to the stain. This temporary stain is desirable for locating the blastocyst in the paraffin block. The blastocyst is then carried to absolute alcohol,

the changes from 70% differing by 5%. At this time the bulk of the alcohol is removed and by gentle rotation the inside of the dish is coated with glycerin. Cedar oil is then substituted for the alcohol and the blastocyst is left in the cedar oil until one hour after it sinks (overnight is convenient). A precaution to be observed at this point is that the dish should be filled only one-half to two-thirds with cedar oil. A full dish seems to inhibit the normal "sinking" of the material.

The cedar oil is washed out with a 2-3 minute change of xylol and replaced with paraffin-xylol for 2-3 hours. Three changes of paraffin-xylol (24 g. per 100 cc.) are made during this time. Five changes of paraffin (56° C.) are made during the 1½ hours of infiltration. The bulbs are removed from the pipets and the pipets placed in the warm oven between changes. The microscope is placed near the oven so that the changes can be made quickly and effectively.

At the time of embedding the blastocyst, the embryological dish is placed inside a Petri dish containing ice-water. By means of a hair mounted in a hemostat the blastocyst is raised gently until a film of solid paraffin has formed over the bottom of the dish. The blastocyst is allowed to rest on the paraffin and oriented into position; when the paraffin has hardened, the block may be marked with a needle to indicate the position of the blastocyst. A marker in the paraffin is not necessary because of the temporary staining of the blastocyst.

The blastocyst is then sectioned as described for the egg. The ribbon is observed under the microscope to guard against wrinkles in the tissue. The sections are destained and then stained permanently with Heidenhain's iron hematoxylin.

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A HISTOLOGICAL STAIN FROM THE BLACK WALNUT (*JUGULANS NIGRA* L.)

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Greenville, Pa.*

The aim of this work was to investigate the dye found in the hulls of the black walnut (*Jugulans nigra*) and to determine its value as a histological stain.

So far as the authors have been able to ascertain there have been no previous investigations in this field altho references have been found which indicate that the black walnut might be a possible source of dye material. During the early history of this country homespun goods were often dyed with the extract of walnut hulls. The extract was obtained by boiling some of the blackened hulls in water. When goods were boiled in this extract they took on a good brown color which did not fade or run. Altho this seems to have been quite common practice in this country and Europe, apparently there has been no investigation made as to the possibilities of this dye material as a histological stain.

It was the opinion of the authors that if certain tests were made upon the properties of the walnut dye before attempting to do any actual staining, they would greatly facilitate the work which followed. For example, in order to prepare staining solutions and also to obtain a pure dye, the action of various solvents was studied. In experimenting with various mordants much time was saved by testing the ability of the dye to react with various chemicals before actually trying them as mordants. It was also the intention of the authors to attempt to determine the structure of the dye, but on account of the difficulties encountered and the time available it has been necessary to defer such investigation until a later date.

The method of testing the solubility of the dye consisted in extracting 0.5 g. portions of the ground, dried walnut hulls with 100 cc. portions of various organic and inorganic solvents. The extraction process was continued for 24 hours and was accompanied by occasional agitation of the liquid. Distilled water was found to be the best solvent for extraction of the dye material. Two parts of distilled water to one part of the dried, powdered hulls were used. Extraction was carried on for 24 hours and the resulting liquid was filtered. When this liquid was evaporated to dryness, over a steam bath, the residue was a hard, black, shiny, crystal-like substance with

a caramel-like odor. A stock solution of the dye was then prepared by saturating distilled water with the dye residue. It was found necessary to add a few thymol crystals to this stock solution to preserve it.

Experimental procedure, using standard methods of staining on the slide, showed that good results could not be obtained by using the stock solution or any of its dilutions. It was found that a mordant was necessary to have the stain "take". Of the various mordants tried, ferric alum was found to produce the best results.

The use of aqueous solutions of the dye extract following the mordanting of the sections for 1 hour in a 5% solution of ferric alum, shows the following results:

The stain is a nuclear stain producing a brown coloration in the chromatin material.

The stain is slow in action. Best results were obtained by the use of the stain for 24 hours.

Some tissues were not overstained by being left in the staining solution for 7 days.

Best results were obtained following the use of picro-formol, picro-sulfuric, and mercuric chloride fixatives.

THE VAPOR METHOD OF CHANGING REAGENTS AND OF DEHYDRATION

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In making permanent preparations from aceto-carmine smears the distortion and shrinkage formerly met with have been largely eliminated by a differential vapor pressure method, briefly described below. This method could have valuable extensions to other materials and reagents and to other fields.

The slide bearing the prepared smear should have a minimum of liquid, with the cover glass chosen as small as feasible, e. g., 15, 18, 22 mm. squares for single pairs of salivary glands and 22 x 40 mm. (18 x 40 mm. if obtainable) oblongs for four or more pairs to the slide. This preparation is put directly, without sealing the edges, into a tightly covered jar kept saturated with vapor of 95% alcohol. The jar should be at least 4 inches deep, preferably 6, and of bottom area proportional to the number of slides to be handled at once. The walls and the cover (of large jars) are lined with wick material—blotting paper or layers of paper toweling or coarse filter-paper, and the bottom is covered more thickly than the sides. This material is kept soaked with 95% alcohol with a slight excess of free liquid. The slides are stood on end around the walls with their lower ends on the very wet paper on the bottom. When the jar is covered tightly the interior soon becomes saturated with alcohol vapor. The alcohol slowly condenses on the surfaces of the slide, mingles with the aceto-carmine at the edges of the cover and seeps under. Displacement occurs progressively thru a slight drainage at the bottom of the slide and thru evaporation into the acetic-free atmosphere which is relatively dry with respect to water. Absorbers for the materials to be removed by evaporation could be used to hasten the process (e. g., CaO for H₂O and acetic acid).

Twenty-four hours is usually sufficient for the dehydration, but no harm follows from leaving the slides several days, providing the excess of free liquid is maintained.

After the vapor treatment the slides are immersed in liquid 95% alcohol in Coplin jars or staining trays and left some hours or days.

The covers are then removed by laying each slide on its back in a bath of 95% alcohol in a shallow Petri dish, and slipping under one corner or edge of the cover glass a thin wedge-shaped needle tip.

Meanwhile the opposite corners of the cover glass should be firmly held in place with the fingertips to prevent the least slippage. When the cover glass has been lifted free and the space below has flooded with alcohol, the cover is removed and cleaned of any adhering material.

Practically all of the material adheres to the slide if the slide has been pretreated with a very thin dried film of albumen as advised by Dr. Hans Bauer.¹ The albumen solution is made by mixing together 100 cc. distilled water and 25 g. powdered egg albumen (Merck). After the excess of albumen has settled out, the supernatant liquid is decanted, diluted to 200 cc. and preserved by 1 g. thymol. A drop of the solution is spread evenly and thinly over the entire slide by scraping with the end of a second slide whose edge is smooth and unchipped.

The slide is lifted from the bath, drained and laid on its back. The material is covered by two or three drops of thinned euparal and the cover is replaced, avoiding bubbles. This stage should be carried thru rapidly in a dry atmosphere without breathing on the mount. The excess of thin euparal is squeezed out between the folds of a paper towel.

No other reagents beyond 95% alcohol and euparal are needed for perfect results, absolute alcohol and xylol being eliminated from the process, but other dehydrants such as dioxan or suitable higher alcohols can be used instead of the ethyl alcohol. "Diaphane" (which can be obtained from Will Corporation, Rochester, N. Y.) can be used instead of euparal in mounting directly from 95% alcohol and is probably better.

¹Bauer, Hans. Notes on permanent preparations of salivary gland chromosomes. *Drosophila Information Service*, 6, 35-6. 1936. Carnegie Institution of Wash., Department of Genetics, Cold Spring Harbor, N. Y.

PERMANENT POLLEN TUBE SLIDES WITH THE VAPOR METHOD OF CHANGING REAGENTS AND DEHYDRATION

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Cold Spring Harbor, N. Y.*

Pollen tube studies from ovaries of *Reseda* species (mignonette), in the family Resedaceae, mounted in a temporary medium such as lactic acid, can be made permanent mounts in balsam with little change in the tissues due to shrinkage or other distortions by the use of the vapor method of changing reagents and dehydration.¹ The use of dioxan² in the vapor jar fixes the pollen tubes embedded in ovary tissue and replaces the lactic acid with dioxan, and prepares the material for permanent mounting in balsam.

The procedure for preparation of pollen tube slides was devised by Buchholz³ and is used for pollen tube studies on *Datura* at the present time. Modifications in technic were made for *Reseda* species, because these ovaries lack a style or stylar tissue in which pollen tubes are generally found. In Resedaceae the pollen tubes grow along the ovary wall, thru parietal placenta tissue. This morphological feature made it necessary to prepare permanent mounts, since the pollen tubes are easily destroyed and moved out of place when mounted in a temporary medium such as lactic acid.

Pollinated flowers are taken into the laboratory where the ovaries are removed and placed in water heated to about 75° C., and kept there for 20 or 30 seconds. The ovary is then removed to killing fluid of formalin and alcohol (6% formalin, 50% alcohol) where it remains for about an hour. After fixation in the killing fluid, each ovary is transferred to water for washing and dissection. The ovaries are dissected under a microscope into the respective carpel parts composing the ovary, which is generally three in the case of *Reseda* species. The carpel possesses a parietal placenta with numerous ovules attached to it, and a stigmatic surface upon which the pollen collects and germinates.

Following fixation, dissection and washing, the carpel was placed

¹Bridges, Calvin B. 1937. The vapor method of changing reagents and of dehydration. *Stain Techn.*, 12, 51.

²Dioxan, chemical solvent secured from Carbide and Carbon Chemicals Corp., 30 East 42 Street, New York, N. Y.

³Buchholz, J. T. 1931. The dissection, staining and mounting of styles in the study of pollen-tube distribution. *Stain Techn.*, 6, 13-24.

in stain. A stain used by Buchholz composed of 80 cc. of a 1% aqueous solution of acid fuchsin and 20 cc. of a 1% aqueous solution of light green SF yellowish was employed in this work. The material was kept in stain for about 10 minutes, washed in water and transferred to lactic acid.

Lactic acid softens the tissue and clears it. This process of clearing and softening takes about 12 hours, after which the entire carpel is transferred to a clean slide with excess lactic acid added to insure proper mounting with the cover slip. A No. 1 or No. 2 cover slip is placed on the material which is spread out on the slide by application of pressure on the cover slip with a needle. This flattening process is done under a microscope to determine the amount of pressure necessary to show the pollen tubes which are embedded in ovary tissue. When good views of pollen tubes are available the slide is ready for permanent mounting.

The slide containing the pollen-tube material, which is mounted in lactic acid, is placed in a dioxan vapor jar. The dioxan vapor penetrates between the slide and cover slip, fixes and hardens the pollen tubes and ovary tissue, and replaces the lactic acid with dioxan. This process of reagent change and dehydration preserves the mounted objects in almost the exact position secured after pressing out the material in lactic acid. There is practically no distortion or shrinkage in the material due to the change of reagents.

Slides are kept in the vapor jar for about 8 hours and then transferred to a jar filled with dioxan liquid. The slide is kept in the liquid dioxan for about 5 or 6 hours. One should not leave the slide in dioxan for too long a period on account of slight destaining which is not desired if the slide is not originally over stained.

The cover slip often comes off in the liquid. Removal of the cover slip by use of a needle, however, does not injure the tissue on the slide. The slide is transferred to a Petri dish where the cover slip may be removed and then mounted in balsam or euparal.

The use of a very thin dry layer of egg albumen on the slide before preparation of the mount, as suggested by Dr. Hans Bauer and described by Bridges, (see footnote 1), will hold all material to the slide.

Permanent pollen-tube mounts have been kept for several months with no apparent change in the tissues. Slides may be filed away and kept for future reference just as any permanent mounted specimen.

IMPROVED SERIAL SECTIONING OF YOLK-RICH MATERIALS

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The preparation of thin serial sections of yolk-rich materials, such as the eggs of certain vertebrates and invertebrates, is notoriously precarious. The heavier amphibian zygotes are rarely cut less than 10 μ thick; even then the yolk-rich portions are usually badly distorted or lost. The rubber-asphalt-paraffin method is of some aid, but by no means overcomes the difficulty.

The writer has found it necessary to prepare serial sections, varying from 4-25 μ , of several hundred zygotes and other early stages of the Pacific coast newt, *Triturus torosus*. It seems quite probable that many others have discovered the method described below. It was never communicated to me, however, altho the problem has been continually discussed with persons interested in microtechnic, nor has it been encountered in the literature. Apparently, then, it is not generally known.

The zygote of the Pacific coast newt is about 2.5 mm. in diameter, seen from the top. It is extremely rich in yolk and presents the difficulties usual to such materials. I have experimented with (1) the addition of rubber and beeswax to the paraffin, (2) paraffins of varying degrees of hardness, (3) mixtures of soft and hard paraffins, (4) short and prolonged treatment in the alcohols, xylol, and paraffin. Only by alteration of a purely mechanical detail, however, have satisfactory thin serial sections been obtained consistently.

To cut sections 4 or 5 μ thick I use 60-68° paraffin; for 8 to 12 μ a 55-57° paraffin is desirable. Dehydrate thoroly, pass thru xylol, xylol-paraffin, paraffin, and embed in the usual manner. Success in sectioning the material depends upon the manner in which the microtome wheel is operated. *The customary uniform rate of rotation, whether fast, medium or slow, is the source of difficulties.* The secret lies in two points: (1) The microtome wheel is rotated rapidly thru almost a complete cycle, and stopped abruptly just as the paraffin block strikes the knife. The sudden contact produces a very firm adhesion of successive sections. (2) The remainder of the cycle is accomplished very slowly; during this time the knife is passing thru the object. This abrupt change of rate is easily accomplished: the rapid part of the cycle is terminated by allowing the wrist or hand to come in contact with the table. A slight bounce is inevitable but this

does no harm; the firm attachment of the sections has been accomplished and the object itself has not yet been reached. The very slow rate at which the knife passes thru the object hardly compresses the paraffin block at all, and reduces to a minimum all distortion of form and displacement of yolk granules. This whole process is mastered within a few minutes and takes much less time than one might imagine, particularly since the major portion of the cycle is soon performed with extreme rapidity. The only rival of this method that I know is to paint the face of the paraffin block with celloidin as each section is cut, a process far more tedious.

Unless particular circumstances dictate otherwise, *the portion of the material poorest in yolk content (e. g., the upper dark hemisphere of amphibian eggs) should strike the knife first.*

RAPID EMBEDDING WITH HOT LOW-VISCOSITY NITROCELLULOSE

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ABSTRACT.—A rapid method for embedding with low-viscosity nitrocellulose is described. Advantage is taken of the greater penetrating power of low-viscosity nitrocellulose than that of common varieties of celloidin. Fixation, dehydration and infiltration are carried out in screw-topped jars in the incubator at 56° C. The whole procedure from fixing to sectioning can be finished in 15–30 hours, and sections as thin as 6 to 10 μ may be cut without difficulty.

For about ten years celloidin sections have been used extensively in this laboratory for teaching purposes; in the hands of beginners the celloidin method proved easier to handle, and yielded preparations superior to those prepared by the paraffin method. But a serious objection to the celloidin technic was the days or even weeks required; this frequently determined the use of the paraffin method. Wetmore (1932) and later Walls (1932) described a more efficient celloidin technic based on Jeffrey's original method; Walls gave an excellent description of the hot celloidin technic employed in his laboratory. It was found possible to use a temperature of 45–60° C. during the infiltration and thickening processes without injury to animal tissues; the pressure produced by evaporation of the solvent facilitated the penetration of hot celloidin into the tissue. It was also found that the number of concentrations of celloidin used in the process of infiltration might be limited to three or five. Ruby (1933) introduced into this laboratory the use of low-viscosity nitrocellulose as an embedding medium; since then our attention has been directed toward simplifying the technic, especially toward reducing the time required. Davenport (1934), working with low-viscosity nitrocellulose, noticed that even "22% solution of the low-viscosity material was sufficiently fluid to penetrate after direct transfer of tissue from alcohol-ether to it without using intermediate concentrations." Results in this laboratory agree with Davenport's observation, and indicate that low-viscosity nitrocellulose penetrates better than celloidin. The attempt further to reduce the time required for infiltration by using low-viscosity nitrocellulose at high temperature has proved so successful that this method has almost supplanted the paraffin technic in this laboratory.

METHOD

A. Fixation. Fix pieces of tissue, not thicker than 2-3 mm., for an hour at 56° C., or 2-5 hours at 37° C., in 10% neutral formol, Bouin, Susa or Carnoy II. (Screw-topped jars were used when the tissue was treated in the incubator during any step of fixation, dehydration or infiltration.)

B. Washing. Wash in several changes of distilled water, one hour each at room temperature. In case of fixation in Carnoy II, treat the tissue for 2 hours at 56° C., with 3-5 changes of absolute ethyl alcohol to remove the chloroform.

C. Dehydration. This should be carried out at 56° C., as follows:

1. 70% ethyl alcohol, 2 changes half an hour each (iodized alcohol for half an hour if tissue was fixed in fluid containing mercuric chloride).

2. 80% ethyl alcohol, 2 changes half an hour each.

3. 95% " " " " " " " "

4. Absolute ethyl alcohol, 2 changes half an hour each.

5. Absolute alcohol and ether mixture, one hour.

D. Infiltration with Nitrocellulose.¹ Make solutions of nitrocellulose in 3 different concentrations as follows:

For the 10% solution, 10 g. of nitrocellulose (already moistened with alcohol for shipping purposes) is treated with 50 cc. of absolute ethyl alcohol, and then 50 cc. of ether is added.

For the 25% solution, 25 g. of nitrocellulose, 45 cc. of alcohol and 55 cc. of ether are used.

For the 50% solution, 50 g. of nitrocellulose, 40 cc. of alcohol and 60 cc. of ether are used.

It is advisable to make several liters of each concentration, and to allow ample time for impurities to settle.

Infiltrate at 56° C., as follows:

1. 10% solution, one hour.

2. 25% solution, overnight.

3. 50% solution, 2-3 hours.

E. Embedding and Hardening. Transfer pieces of tissue directly to a fiber block moistened with alcohol-ether. Place enough 50% nitrocellulose around the tissue to ensure good embedding. Then immerse the blocks for about one hour in 2 changes of chloroform for

¹Nitrocellulose R. S. 1/2 second, viscosity 3/20-4/20 (A product of the Hercules Powder Company, Parlin, N. J., was used.) The authors wish to thank the Hercules Powder Company for their generous donation of nitrocellulose during the early stage of the development of this method.

hardening, and finally carry them through 3 changes of 80% ethyl alcohol within one hour.

F. Cutting. Section blocks of tissue while wet with 80% alcohol.

In some cases it was found necessary to introduce a slight modification of the technic, especially when sections of 25–100 μ were desired. To make thick sections more pliable during the cutting and subsequent handling, the 50% solution of nitrocellulose was omitted, and the final infiltration and embedding was done in 25% nitrocellulose. It was sometimes helpful to wrap a strip of gummed paper around the fiber block, letting the paper protrude sufficiently to retain the necessary amount of nitrocellulose. With some tissues, such as liver, adrenal, pancreas or spleen, the infiltration time could be reduced to 3 or 4 hours.

RESULTS

The hot low-viscosity nitrocellulose method described herein requires much less time than the standard celloidin method; indeed, it is only slightly slower than the paraffin technic. The whole procedure of fixation, dehydration, infiltration and embedding can be accomplished within 30 hours. When only thick sections are desired, it is possible to reduce the time still further. In many instances animal material obtained and fixed in the morning was sectioned late in the evening of the same day. Neither distortion of tissues nor perversion of staining properties resulted from the speed of the procedure or the high temperature used. The method was tried on a considerable variety of human and animal tissues, and was found applicable likewise to pathological material. A considerable number of such tissues, including a large series of testicular tumors, gave invariably good results. The rapidity of the method should make it practicable for the pathologist, and in students' work, which usually calls for a limited number of sections. No difficulty was encountered in cutting 6–10 μ sections; it has even been possible to obtain serial sections at 5 μ , and in some instances as thin as 3 μ .

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RECENT ADVANCES IN MICROTECHNIC. I. METHODS OF STUDYING THE DEVELOPMENT OF THE MALE GAMETOPHYTE IN ANGIOSPERMS

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ABSTRACT.—Among methods used for a study of nuclear details in the development of pollen grains, the following were found to be very satisfactory: (1) warming the entire grains in aceto-carmine and then clearing with chloral hydrate; (2) making smear preparations stained with crystal-violet-iodine or iron alum hematoxylin. For paraffin sections, a counterstain with dilute alcoholic erythrosin is often very useful after the usual iron hematoxylin technic.

A method of making cultures of pollen tubes on slides coated with thin films of sugar agar is described in detail. The tubes can be fixed by immersing the slide in formol-acetic-alcohol and then stained by any desired schedule. Iron alum hematoxylin was found to be the most satisfactory, but the Feulgen reaction is very valuable in such cases where the nuclei are obscured by the density of the pollen tube cytoplasm. Living pollen tubes can be kept under observation by dissolving a small quantity of neutral red or other vital stain in the sugar agar before it is spread on the slide.

For studying stages in fertilization or gametogenesis, styles should be fixed and sectioned only after a preliminary study with iodine-chloral-hydrate or safranin-anilin-blue or aceto-carmine. Once the extent to which pollen tubes grow in a given time in the stelar tissues has been determined, it is possible to fix material with some knowledge of what it is going to show.

Some other methods, that have not been tried by the authors but appear to be valuable, are also briefly described.

Even a cursory glance at the literature on the embryology of the Angiosperms shows that there are many more works on the ovule and embryo sac than on the male gametophyte. Indeed, statements on the latter are often limited merely to a count of the number of nuclei within the pollen grain.

Recent advances in microtechnic have made it possible to follow the whole history of its development, from the time of the first division of the microspore up to the formation of the male gametes,

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with greater precision and ease. It is the object of this paper to summarize this literature here and to describe the more valuable of these methods in sufficient detail to encourage others to make use of them.

POLLEN GRAIN

The customary technic of making paraffin sections of the anthers still holds its place, but it is often possible to make observations of value by simpler means. In a recent paper Wunderlich (1936) writes that she was able to see the generative cell in *Haworthia*, *Tulipa*, *Ornithogalum* and *Fritillaria* by just mounting the grains in water without adding any stain whatever. To these we can add, from our own experience, *Hippeastrum hybridum* and *Agapanthus umbellatus*. It is worth mentioning that Meyen (1839), who was the first to see the generative cell, found it in the same genus (*Fritillaria*) and under the same conditions as Wunderlich did nearly one hundred years later.

It must be admitted that such a condition is only rarely met with, but there is another ready method near at hand. All that is to be done is to mount a few pollen grains, fresh or preserved, in a drop of aceto-carmin (made according to Belling's formula), put on a cover glass and warm gently over a flame, adding more aceto-carmin from the sides if necessary (see Heitz and Resende, 1936). Unless the exine is too thick, it is possible to see the generative or sperm nuclei and sometimes even the plasm around them quite clearly. It is often useful to add a few drops of a 10% solution of chloral hydrate (Satina and Blakeslee, 1935), since this clears the cytoplasm from inclusions which otherwise obscure the nuclei or chromosomes. Experience has shown that in some cases when the nuclei or spindle are not readily visible just after warming in aceto-carmin, they can be seen a few days later in the same preparation. If it is desired to save the preparations for some time longer, they can be sealed with a mixture of gum mastic and paraffin.

The following method, used by Warmke and Johansen (1935) to study the first mitosis in the microspores of *Trillium*, may be tried in those cases where the exine is too thick and does not permit an observation of the contents of the grain²:

1. Kill in 1 part glacial acetic acid and 3 parts alcohol.
2. Transfer to 1 part 95% alcohol and 1 part conc. HCl for 5-10 minutes. The HCl causes the exine to split and fall away like the shell of a nut leaving the naked protoplast adhering to the slide.

²Our own trials, made with pollen grains of some Dicotyledons were entirely unsuccessful. It seems likely that this method will work best with such plants in which the pollen grains have a single prominent furrow.

3. Transfer to a drop of Carnoy's fluid to harden the material.
4. Stain in aceto-carmine in the usual way.

In many cases, where the microspores are surrounded by a viscous fluid in the anther (viz. some Commelinaceae) it is possible to make smear preparations to study the first mitosis in the pollen grain. The usual procedure described by Taylor (1924) answers the purpose admirably, but it is often better to stain in Newton's gentian violet (see Sax, 1931), since this gives greater transparency to the cytoplasm and makes the chromosomes more conspicuous.

It is always useful to check the results by making paraffin sections also, but instead of cutting a large number of anthers at random, it is possible to save time by fixing only material of the required age after a preliminary examination by one of the methods described above. Thus, if it is desired to prepare sections showing the first division of the microspore in *Tradescantia*, we should examine anthers from several flower buds in aceto-carmine. When an anther from a bud does show the required stage, the remaining anthers should be fixed immediately.

Good fixation and a critical stain with iron hematoxylin will reveal the presence of generative and sperm cells in many plants where only nuclei have been reported in the past. A counterstain with erythrosin sometimes makes the effect more sharp. Cooper (1935) who studied the development of the male cells in *Portulaca oleracea* recommends Ehrlich's hematoxylin because of its transparency. In those cases, where the nuclei become obscured by the presence of large amounts of food substances in the pollen grains, the Feulgen reaction often yields fine results since only the nuclei are stained by this method.

POLLEN TUBES IN CULTURE

Making cultures of pollen tubes is usually quite easy. Some pollen grains germinate in distilled water without the addition of any other substances (as in grasses). It is also possible to induce germination of pollen grains *in situ* (i. e., in the anther loculus itself) by corking up the flowers in a tube containing some water at the bottom.

Schleiden (1842-43) was the first to try sugar solutions of different strengths for the germination of pollen grains. Kny (1881) was impressed by the similarity of growing pollen tubes and fungus hyphae and began to add gelatine. Trials by other workers demonstrated the superiority of agar-agar³ and Jost (1907) made his cultures on

³It is only in rare cases that agar inhibits the growth of pollen tubes. Thus, Medwedewa (1935) reports that in *Apocynum venetum* the growth of the tubes stopped entirely on the addition of agar to the sugar solution.

slides coated with a thin film of sugar agar. ✓ Herrig (1919) germinated pollen grains of *Butomus* and *Echeveria* on agar but he later (1922) cut it up into blocks which were sectioned after embedding in paraffin. This seems to be quite unnecessary since the contents of the pollen tube can be seen quite clearly thru the wall and with sections there is the further disadvantage of having only fragments of the tubes in view and not their entire length.

In recent years Cheesman (1927) records having obtained a good growth of pollen tubes of *Theobroma cacao* on a thin film of sugar agar spread on the slides. He fixed them in Bouin's fluid and used iron hematoxylin for staining.

Trankowsky (1931) perfected the method and tried it on several plants with great success. Since then Wulff (1933, 1934a, 1934c, 1935), Poddubnaja-Arnoldi (1933, 1934, 1936), Safijowska (1935), Fuchs (1936), and others have made considerable use of it. The following outline will, it is hoped, give sufficient information to enable any one to obtain good preparations of pollen tubes showing the male cells and vegetative nucleus, (using, for example, *Impatiens Holstii* or some other species of this genus as a trial object):

1. Culture solution: For every 100 cc. of distilled water, weigh out 1 g. of agar-agar and 2.5, 5, 7.5, and 10 g. of sugar. Plug the tubes with cotton wool and leave in an autoclave or on a water bath till the agar dissolves. These concentrations will suffice for most objects, but sometimes 15-50 g. of sugar may be necessary.

2. Clean slides first with conc. HNO_3 or a mixture of potassium dichromate and sulfuric acid, then rinse in water and rectified spirit and finally wipe them dry with a clean cloth, free from lint. It is necessary that the slides should be absolutely clean because the agar will not spread on dirty slides, and even if it is made to do so it may come off afterwards during the process of fixing and staining.

3. If the culture solution has set, warm it again on a water bath till it becomes perfectly fluid. Flood the right half of the slide with this and then shake off all the surplus sugar agar, so as to leave only a very thin homogeneous film on the slide. If the film is too thick, it will tend to come off at a later stage or it may itself take so much of the stain that the mount will be rendered useless. This film can be spread on half a dozen slides which are then laid flat on a white paper. (A solution containing 1 g. of agar and 2.5 g. of sugar is quite satisfactory for *Impatiens Holstii*.)

4. Let the agar cool for a minute or two and then sprinkle the pollen by means of a sable brush. The flowers must, of course, be kept ready beforehand, each with some half-opened anthers. Immature pollen grains may not germinate. The usual precaution of sterilizing the brush before each attempt with a different plant must not be forgotten.

5. Now keep the slides in a moist chamber maintained at a temperature of 15–22° C. and watch from time to time to see if germination has taken place. In *Impatiens Holstii* 30 or 40 minutes are enough for the division of the generative cell in the pollen tube and it is easy to get many mitotic figures on a single slide. In detailed studies of the division of the generative cell material must be fixed from time to time so as to get all stages.

The aceto-carmine method is particularly useful at this stage and enables a rapid glance of the nuclei in the tube. Just add a few drops of the solution on the slide and put on the cover slip. Warm on a heated brass plate (the time can be determined only by experience) and examine under the microscope. If the nuclei are not clearly seen, add more aceto-carmine from the sides and heat again. Permanent mounts can be made by following the procedure recommended by McClintock (1929) for smears of pollen mother-cells. Steere's method (1931) is not practicable in this case since inverting the slides in hot aceto-carmine loosens the agar film from it. The disadvantage in the aceto-carmine method is that often the vegetative nucleus does not stain at all and the generative plasm around the generative or sperm nuclei is also invisible.

6. For the finest preparations, it is best to fix the cultures and then stain in iron haematoxylin. The slides may simply be immersed in a Coplin jar containing formalin-acetic-alcohol (formalin, 5 cc.; glacial acetic acid, 5 cc.; 50% alcohol, 90 cc.) or Pfeiffer's fluid (equal parts of formalin, methyl alcohol and crude acetic acid). Nawaschin's fluid or chrom-acetic acid may also be used, especially if gentian violet is to be used for staining. The only disadvantage is that the pollen tubes do not hold so well on the slides after fixation in aqueous fluids and sometimes they burst during the process of fixation. The latter difficulty can be overcome (as suggested by Trankowsky) by first fixing for 2–3 minutes in a solution containing the same percentage of sugar that was used in the culture. After this preliminary killing, the slides can be transferred to another jar containing the fixing fluid made up according to the original formula. The time of fixation should be about three hours.

7. Wash the fixative with water or alcohol as the case may be. Only half a dozen changes will be enough. Let the slides remain in the jar and allow a gentle stream of water to flow in from one side. Careless handling at this stage may cause the agar film to get loose from the slides.

8. For staining follow the usual procedure with iron alum hematoxylin. About 10 minutes of mordanting and 10 minutes of immersion in hematoxylin will be sufficient. Differentiate in a saturated aqueous solution of picric acid or 2% iron-alum. Use distilled water for washing and pass thru 30, 50, 70 and 90% alcohols to absolute alcohol. Before passing into pure xylol it is advisable to use one grade of a mixture of absolute alcohol and xylol. Mount in thin Canada balsam. The generative nucleus will be well stained and its cytoplasm will be distinguishable from the cytoplasm of the tube.

Newton's gentian-violet-iodine method will also give good results if the tubes are broad and the nuclei large, but it is necessary that fixation be carried out in a fluid containing chromic acid. The disadvantage of this method is that the cytoplasm around the generative nucleus usually remains indistinguishable from the general cytoplasm of the tube.

The Feulgen reaction (Wulff, 1933) can be made use of in special cases to ascertain whether the vegetative nucleus, which is often difficult to see even after staining with hematoxylin, is still present or has actually degenerated (or fragmented). Many of the pollen tubes get washed away during the period of hydrolysis in warm HCl, but when sufficient care is exercised some can always be retained on the slide and give valuable results for comparison.

Vital staining. This is of some importance since it enables observations on living pollen grains and pollen tubes. Wóycicki (1926a, 1926b) was the first to show that in *Haemanthus Katharinæ* the plasm of the generative cell contains a vacuome which can be seen after staining with neutral red. Since then it has been seen in several other Monocotyledons (Wulff 1933) and in such cases this method can be used not only to demonstrate the presence of the generative plasm but also in following the passage of the generative or sperm cells, which stain red when pollen tubes are grown on a sugar agar substrate containing a small quantity of neutral red in solution. Similar results have been reported with chrysoidine (Wulff, 1934b) but we do not yet have enough observations of this kind to say in how many cases it will actually succeed. A brief mention may also be made of the work of Breslavetz who used "Janusschwarz" to stain pollen tubes of *Cylamen* and found the sperm nuclei to be surrounded by a lighter zone while the mitochondria, which were seen only in the vegetative plasm, took a black stain. Finally there are some exceptionally favorable plants like *Agapanthus umbellatus* and *Hippeastrum hybridum* where the generative cell can be seen in the pollen tube without any staining whatever.

POLLEN TUBES IN STYLE

While pollen tubes can be grown quickly in the way described above and all stages in the division of the generative cell (unless it has already taken place in the pollen grain) obtained by fixing them at regular intervals, this method can not entirely take the place of observations made on tubes growing in the style. As shown by one of us (Wulff, 1935) the mechanism of division of the generative cell

may show important differences under the two conditions and a comparison is always useful.

In this case also, a great deal of time and labor can be saved by making some preliminary observations before fixing the material for embedding it in paraffin. In the case of slender styles, it is possible to determine the presence of pollen tubes merely by staining the entire styles in iodine and clearing with chloral hydrate. Those parts of the tubes which contain starch stain black, while the starch-free portions stain dark yellow. If permanent preparations are desired, the entire styles can be fixed in formalin-acetic-alcohol or Carnoy's fluid and stained in eosin after which they can be dehydrated, cleared and mounted in balsam. A combination of safranin and anilin blue is also suitable since this stains the callose of the wall of the tube and makes it conspicuous. Better still is to stain in aceto-carmine, dehydrate with alcohol, clear with clove oil, then rinse with xylol and finally mount in balsam. Poddubnaja-Arnoldi, Steschina and Sosnovetz (1935), who tried this method in their work on *Scorzonera tausaghyz*, report that the pollen tubes alone took the stain while the other tissues of the style remained practically colorless, so that it was possible to trace their course very easily and in some cases even the sperm nuclei were visible.

In the case of thicker styles, free-hand sections can be made and stained by one of the methods given above. Nebel (1931) recommends crushing the styles between two slides and then staining with 5 mg. of lacmoid and 5 mg. of martius yellow dissolved in 10-15 cc. of water. Anderson (1936) smeared the styles on a slide at various intervals after pollination and then stained with crystal violet. Pollen tubes could thus be traced right down to the micropyle, after which the ovaries were fixed at 2 minute intervals until fertilization had occurred.

Another method (Schoch-Bodmer, 1932), which can be tried for long styles, is to pollinate the stigma, cut it off with a small part of the style attached to it and keep the piece in a moist chamber. The pollen tubes will emerge out of the cut end after some time. The conditions requisite for success are: a clean cut surface, without a blocking of the intercellular spaces and a proper degree of moisture and temperature. This gives an approximate idea of the rate of growth of the pollen tubes and by measuring the total length of the style in open flowers it is possible to make a rough prediction about the approximate time that will be taken by the tubes to reach the ovary. It is of interest to add that Buchholz, Doak and Blakeslee (1932) made some careful experiments and found that it is possible to shorten

the length of the style by removing a piece from the middle and joining the two cut ends. Even when parts of two different styles were joined and properly held in place, many pollen tubes could pass thru the wounded area.⁴

The presence of the pollen tubes once determined, it is possible to fix material with some knowledge of what it is going to show. Thus, if the division of the generative cell is to be studied, it is best to fix styles at different intervals soon after the pollen tubes have begun to germinate (a preliminary study of cultures stained with aceto-carmin may often indicate the time of the division of the generative cell rather precisely). If the object is to study stages in fertilization, fixation should begin at very close intervals after the pollen tubes have reached the base of the style.

Small and thin styles offer no special difficulty in fixation and can be dropped whole into the killing fluid, but it is best to cut up or split the larger ones and use an exhaust pump to facilitate infiltration. Nawaschin (1910), in his excellent work on *Lilium*, writes that he injected the killing fluid into the styles.

For observation of cytological details there is nothing better than the customary iron hematoxylin technic, but a counterstain with dilute alcoholic fast green increases the visibility of the pollen tubes. If they are relatively broad and the material has been fixed in one of the chromic acid fluids,⁵ Newton's gentian violet and iodine will also give good results. Sieben (1913) gives 2 mixtures of the Planeze stain, and in his work on *Asclepias cornuti*, Finn (1925) recommends this as the best. Without giving further instances it may be stated that some plants react favorably to one method and some to another and the investigator has to find out for himself which is best for his particular needs—first, for locating the pollen tubes and thus determining the approximate time for the fixation of the styles and then for a critical study of the contents.

Finally, a brief mention may be made of cases when it has been found possible to dissect out the pollen tubes from the style and then

⁴Jost (1907) had used an essentially similar method to bring about the opposite effect of lengthening the style. In *Hippeastrum* the style was cut off at the base a day after pollination and then placed in a moist chamber with its lower end in close contact with the stigma of another flower. A week later the tubes had travelled into the second style. The same was accomplished in *Lilium martagon* by bringing the cut surface of the first style in contact with the upper end of the cut surface of a second style. In this case the second stigma was removed since it caused difficulties in the passage of the tubes.

⁵Artschwager and Starett (1934) report that in *Beta vulgaris* the pollen tubes could be located with difficulty in material fixed with Carnoy's fluid but stood out distinctly when Nawaschin's was used.

stain them—an operation whose success depends to a large extent on the structure of the style. Sawyer (1917) found that in *Iris versicolor* each of the 3 branches of the style is traversed by a longitudinal groove covered by 2 overlapping outgrowths, thus forming an inclosed canal with the pollen tubes inside it. By removing the covers of the groove, she could expose the canal containing the tubes and remove them with needles. In some cases it is possible to split the style longitudinally by beginning at the stigma and carrying the cut downward with a pair of fine needles. The pollen tubes can then be exposed by spreading the cut surfaces apart and laying on a clean slide. Further details will be found in the papers by Chandler (1931) and Buchholz (1931).

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NOTES ON TECHNIC

A CELLOPHANE EMBEDDING BOX.—A new paraffin embedding technic which may be of interest to others is being successfully used in this laboratory. It involves the use of embedding boxes made of No. 450 M. T. cellophane instead of paper boxes. They are made by cutting and folding the material just as paper boxes are made. This new method has these advantages:

1. The fixed material is dehydrated, cleared, and paraffin is added, without leaving the cellophane box.
2. The transparent box allows the smallest material to be observed easily under the dissecting microscope.
3. The cellophane separates from the paraffin cleanly.

Pipets with annealed tips are recommended for handling small material when using this technic.—F. F. FERGUSON, University of Virginia, University, Va.

A TECHNIC FOR STAINING CELLS WITH SUDAN III IN A WATER PHASE.—Since Sudan III is not soluble in water its use is attended with some difficulty. It is generally employed in a solution of alcohol, or alcohol and glycerin. There is an objection to these solvents, especially when one wishes to study both the lipidic features and the hydration properties of the intravacuolar spheres. A solvent is needed which will carry Sudan III in an aqueous phase into the cell, without the previous use of killing agents.

Spherical inclusions of lipoidic material in the cell vacuole are among the most important indices of impaired physiological activities of the plant cell. We have shown that the hypoplastic cells of orange leaves affected with mottle-leaf commonly contain these highly refringent spheres of phytosterol material, mainly in the epidermal or adjoining palisade layers.¹ Similar bodies are to be found in cells of various plants in the families of Solanaceae and Compositae when affected with the "spotted wilt". These spheres stain readily with fat soluble dyes, specifically with Sudan III; or they may be stained gray with osmic acid in low concentration, or blue with nascent indophenol blue, formed as a suspension in a mixture of alkaline solutions of paradiphenylamine hydrochloride and thymol.

We have found it possible to study the physical properties of these spherical inclusions as a lipid phase in contact with the aqueous phase of the vacuolar sap by the method hereinafter described.

¹Reed, H. S., and Dufrenoy, Jean. 1935. Modification in cell structure accompanying mottle leaf of the orange. *Amer. J. Bot.*, 22, 311-23.

Methylal was used to dissolve Sudan III, and to carry the Sudan III in the form of a suspension into water.

A strong solution of Sudan III was first prepared in 5 cc. of methylal. This was poured into a small vial and 10 to 20 cc. of water added. The water first mixes with the methylal, but in a few minutes the liquids separate, leaving the lower, light orange phase containing water + methylal + Sudan III and an upper phase containing methylal + Sudan III + water.

When free-hand sections of tissue whose cells contain intravacuolar spheres (for instance, leaves of *Callistephus sinensis* affected by the virus of "spotted wilt") were immersed in the liquid, they either sunk to the bottom of the vial, or floated at the interface. In either case they were in contact with a water phase from which they absorbed some Sudan III. As they absorbed dye from the water phase, dye entered from the upper concentrated solution.

Sections were transferred to a slide at the end of 30 minutes and studied under the microscope. The intravacuolar spheres of lipoid material were stained bright orange.

In the course of several hours the methylal may become hydrated to an extent that Sudan III no longer stays in solution, but it remains in solution long enough for the technique above described.—J. DUFRENOY, Station de Pathologie Végétale, Pont-de-la-Maye, Bordeaux, France and H. S. REED, University of California, Berkeley, California.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

BOOK REVIEWS

GAGE, SIMON HENRY. *The Microscope*. 16th ed. 6 x 9½ in., 617 pp. Cloth. 311 illustrations. Bibliography. Index. Comstock Publishing Co., Ithaca, N. Y. 1936. \$4.00.

The sixteenth edition of this well-known book is published 4 years after the preceding. Altho the price has not been changed, the text material of this edition has been increased by 25 pages; also the general appearance of paper, print, and binding has been improved. The major new addition is a chapter on micro-incineration. It includes the technic of reducing sections to ashes in such a way as to permit identification of the various inorganic materials in position; also it offers suggestions as to the best optical equipment suitable for such a type of work. A section discusses fully the coating of mirrors by the aluminum vapor method and their superiority over quartz prism reflectors for ultraviolet microscopy. The bibliography has also been brought up to date.—*J. A. de Tomasi*.

MICROSCOPE AND OTHER APPARATUS

FOOTER, A. W. Simplified preparation of microscope cross hairs. *Science*, 84, 490. 1936.

For some time the author has been using spider silk for cross hairs on microscope oculars; but because of the difficulties in obtaining and using this material, he now proposes an improved procedure. The proposed method relies upon the use of a commercial waterproof adhesive (not specified, but assumed to be pyroxylin). A small globule of this cement is taken on the end of a probe and touched to the surface of the diaphragm of the ocular, after removal of the upper lens. Pulling the probe away some 9-12 in. leaves an extremely fine and elastic thread attached to one side of the diaphragm. The opposite side of the diaphragm is then moistened with the same adhesive and the thread from the first drop of the substance is caught a little short of the length necessary to cross to the other side; it is then stretched out to the requisite extent and secured on the opposite side of the diaphragm by means of the second drop of adhesive.—*H. J. Conn*.

KNISELY, M. H. A method of illuminating living structures for microscopic study. *Anat. Rec.*, 64, 499-524. 1936.

This paper describes a quartz rod method of illumination for studying living organs, with especial reference to circulatory changes including capillary blood flow. Fused quartz rods, if meticulously clean and free from wrinkles and bubbles, conduct the light waves without great loss even around curves to a bent up delivery tip placed beneath the organ to be studied. The fact that quartz is a poor conductor of heat helps in preventing too great conduction to or from the tissues studied. The light source is generally a tungsten filament projection bulb, air cooled and used without condenser lens. At times a condenser made in the form of a glass funnel, externally silvered, was used and the receiving end of the quartz rod placed adjacent to the narrow end of the funnel. The angle at which the light rays enter the quartz rod should be greater than the critical angle of fused quartz so that internal reflection will keep the rays within the rod and

send them toward the delivery tip. The quartz rod is made in two sections so that filters may be inserted between the source and delivery ends. Rack and pinion is used for movements of the delivery tip, and 2 adjustable clamps for the ends nearer the light source. Adequate drawings show the arrangement of the parts. The animals studied are mounted on a 3-legged table free from the microscope. This can be moved without disturbing the delivery tip of the quartz rod. The end of the rod should not touch the tissue. The tip is further cooled by a stream of Ringer's solution against its side. The control of vibration in the apparatus is described. There follows a discussion of the efficacy of the method to demonstrate normal processes arrived at by testing animals under varying conditions of anaesthesia and illumination. Intense light, if properly cooled, did not seem to alter capillary circulation. A summary of the advantages and limitations of the method is given.—*S. I. Kornhauser.*

SCOTT, G. H. and WILLIAMS, P. S. A simplified cryostat for the dehydration of frozen tissues. *Anat. Rec.*, 66, 475-81. 1936.

This apparatus is constructed entirely of metal. The tissue chamber is immersed in a 5-gallon pyrex vacuum flask containing solid CO_2 and butyl alcohol, which produces a temperature of -78°C . From the upper part of the metal casing containing the tissue chamber is an outlet to a Cenco Hyvac pump. A mercury regulator is connected with an alcohol chamber placed directly above the tissue chamber. The alcohol chamber is wound with an electrical heating element by which the chamber may be warmed during the process of dehydration. By means of the regulator, the temperature of the tissue chamber may be varied between -70°C . and 0°C ., the higher temperatures facilitating the removal of the water vapor from the frozen tissues. The cost of operating the apparatus in cool weather is less than 20 cents for 24 hr. A sectional mechanical drawing of the apparatus is given.—*S. I. Kornhauser.*

SHINN, L. E. Protection of eyepieces. *Science*, 84, 400. 1936.

To prevent upper lenses of eyepieces from getting soiled or dusty, a circular cover slip of 20-25 mm. diameter is a convenient cover, when casing rim allows it. It is easily removed and does not interfere with vision.—*J. A. de Tomasi.*

PHOTOMICROGRAPHY

KOCH, W. Eine einfache Vorrichtung zur Mikrophotographie und zur Photographie kleiner lebender Objekte. *Zts. wiss. Mikr.*, 53, 37-42. 1936.

This is substantially a report on the adaptation of a well-known miniature camera to photomicrography. The camera is the "Exacta" made by the Ihagee-Kamerawerke of Dresden, Germany. Its outstanding features are a reflecting mirror for focusing and a focal shutter. Additional equipment suggested consists of a tube extension, a hinge piece connecting the camera to the microscope, and a ring diaphragm.—*J. A. de Tomasi.*

MICROTECHNIC IN GENERAL

EDMONDSON, W. T. Fixation of sessile Rotatoria. *Science*, 84, 444. 1936.

Fixation in a life-like manner of sessile Rotatoria is extremely difficult. The following procedure is suggested: By means of a pipet take the rotifer attached to a small part of its host plant and transfer it to a watch glass. When animal is extended, pour onto it 1-2 cc. of boiling Zenker's solution. Rinse immediately in water. The technic proved to be satisfactory with several genera except *Floccularia* and *Limnias*.—*J. A. de Tomasi.*

HOERR, N. L. Cytological studies by the Altmann-Gersh freezing method. I. Recent advances in the technique. *Anat. Rec.*, 65, 293-313. 1936.

A history of the freezing drying method is first sketched. The author's present methods are then given and finally a statement of the advantages and disadvantages of the method together with cytological illustrations.

Tissues are immersed in iso-pentane chilled to -195°C . by liquid nitrogen. In 15 sec., a piece 5 mm. thick is frozen without producing visible gas bubbles.

For large pieces of tissue 200 cc. of iso-pentane should be used. Liquid nitrogen is the safest refrigerant. Animals dehydrated by starvation for several days show fewer ice crystal artefacts. In the dehydration of frozen tissues a temperature of -20° C. did not prevent formation of ice crystals which resulted from the concentration of salts of tissues during dehydration. In general, -33° C. gave good cytological pictures. For some tissues -60° C. was used. The temperature must be reduced slowly to the dehydrating temperature. In general, rapid dehydration is best and a triple stage mercury diffusion pump, backed up by a Hyvac oil pump, was used. A McLeod gauge and a discharge tube indicated when dehydration was complete. Phosphorus pentoxide aided in obtaining high vacuum. From 0.5-1% of water remains in the tissue and can be removed by desiccation in CaCl_2 at 37° C. Paraffin embedding of dehydrated tissues is held injurious as paraffin and petroleum ether dissolve the highly dispersed lipoids in the protoplasm. Free-hand sections $15-20\ \mu$ can be cut of most tissues. These may be cleared in 1 min., or less in vacuo in a drop of paraffin oil or anhydrous glycerin.

Enzymes are not destroyed by freezing and drying. The main advantage is instantaneous fixation or cessation of chemical activity. Thus the spleen will show structures only seen in life and not seen after ordinary fixation. Vacuoles of various sizes and form are always formed but rapid freezing reduces these to a minimum. The smallest vacuoles are spherical and are believed to be caused by gases making their exit from the tissues.—S. I. Kornhauser.

IWANOFF, X. Über das Aufkleben von Gefrierschnitten. *Zts. wiss. Mikr.*, 53, 48-9. 1936

Frozen sections can be made to adhere to glass by the following procedure: Smear the cover slip with a 2:1 albumen-glycerin mixture and dry for 24 hr. in the upper shelf of the paraffin oven; if desired to avoid this delay, a 1:1 mixture of albumen (or blood serum) and 5% formalin can be used immediately. Transfer section to the slip. Support a few cover slips, sections right side up, with a glass slide. One ordinary slide will hold 4 average size cover slips. Cover with a strip of filter paper the size of the slide, put on another slide, press down, remove upper slide, fix the section by wetting with 1-2 drops of abs. alcohol or 40% formalin, replace second slide and press down gently for 30 sec.—J. A. de Tomasi.

WERTH, S. C. The use of liquid air in cooling knives and of gelatin for mounting in frozen section technic. *J. Lab. & Clin. Med.*, 21, 1309. 1936.

In connection with the frozen section technic two practical hints are suggested. The microtome blade can be immersed without damage in liquid air in a thermos bottle just before using. The effect of this treatment lasts about 5 min. The use of 10% gelatin instead of glycerin eliminates most of the trouble due to bubbles.—J. A. de Tomasi.

DYES AND THEIR BIOLOGICAL USES

HOLLBORN, K. Hämatoxylin (Vereinfachte und verbesserte Hämatoxylin-Simultanfärbungen.). *Zts. wiss. Mikr.*, 52, 184-9. 1935.

Hematoxylin is a crystalline product obtained by a water-ether extraction of the hardwood of *Hæmatoxylin campechianum*. The addition of Fe, Cr, and Al ions changes it to lakes which behave like basic compounds, displaying a specific affinity for the nucleic acid of the nucleus. The following directions are given for the preparation of a number of hematoxylins and hematoxylin compounded stains, all made from Bayer's standardized hematoxylin.

Hæmatein und Hæmatein-Ammon: Use this product by dissolving 1 g. in 1000 cc. 5% alum by heating. Cool and filter. Preserve by adding 10% hartosol, methyl or ethyl alcohol.

Hæmalaun: Dissolve 5 g. in 100 cc. hot dist. water. Cool, filter and preserve as above.

Eisenhämatoxylin "H": Dissolve 2 g. in hot dist. water. Cool and filter. Stain sections 20-30 min. Rinse in water and alcohol. Clear and mount. This can be followed by *Van Gieson-Elastin-Farbstoff "H"* for a multicolor stain.

Nucplascoll: Dissolve 2 g. in 100 cc. hot dist. water. Cool, filter and preserve as above. Treat sections as with Fe hematoxylin. Nuclei stain black; collagenous fibers, green; muscles, red; blood and hairs, yellowish.

Hämatoxylín-Eosin "H": Preparation and staining as with nucplascoll. Nuclei appear black; the eosin gives a multicolor stain.

Hämatoxylín-Sudan "H" Lösung: Supplied only in solution. A simple application will stain nuclei black and the fat red. Blood films or frozen sections can be stained 1 hr. in closed jar. Rinse in 70% alcohol, water and mount in glycerin, glycerin-gelatin, glycerin-rubber, or gelatin-balsam.

All these stains, for which simpler manipulation and saving of time is claimed, are products of the firm Dr. K. Hollborn & Söhne, Leipzig.—*J. A. de Tomasi.*

HOLLBORN, K. Karmin und Cochenille. (Vereinfachte und verbesserte Karmin- und Kernechtrot-Simultanfärbungen.) Zts. wiss. Mikr., 52, 209-13. 1935.

Among many dyestuffs used in staining histological material only one substance is derived from an animal source. It is cochineal or powdered wingless females of the nopal insect (*Coccus cacti*), grown on specially established plantations in Mexico, Brazil, Peru and Java. This powder has a content of about 10% carminic acid. Neither cochineal nor carminic acid have found as wide an application as carmine, a mixture of carminic acid, clay, calcium, and albuminoids. The author lists a number of carmine combinations he worked out in the last decade.

(1) **Pikrokarmin neu I "H":** Stains nuclei red, remaining tissue yellow. Does not require differentiation. Preparation: dissolve 4 g. of dyestuff in 100 cc. hot dist. water. Cool and filter. Stain 30 min.

(2) **Pikrokarmin-Anilinblau "H":** The addition of anilin blue adds blue-stained connective tissue to the picture given by No. 1. Preparation: dissolve 2 g. of dyestuff in 100 cc. hot dist. water. Cool and filter. Stain 30 min., wash with dist. water, pass thru alcohol and xylene into Canada balsam, free from essential oil.

(3) **Multikolor Solution:** Stains nuclei red; elastin brown; erythrocytes and keratin yellow; fat green.

(4) **Nucplastin:** A combination of carmine, acid green, and "elastin stain". Preparation: dissolve 4 g. of dyestuff in 40 cc. hot dist. water on a water bath, 10 cc. glycerin, 50 cc. hartosol or alcohol. Cool and filter. Stain 30 min. in covered jar and refilter before each application. Rinse with hartosol or alcohol; xylene, Canada balsam "H", or "Caedax".

"**Kernechtrot standardisiert, Bayer**" is a synthetic product intended as a substitute for, and claimed to be superior to carmine as a red nuclear stain. It is an anthraquinone dye to be used in 0.1% concentration in 5% $Al_2(SO_4)_3$ solution dissolved by boiling. "**Kernechtrot-Kombination H**" is a compounded product derived from it and gives simultaneous staining of nuclear and connective tissue. Prepare by dissolving 2 g. in 100 cc. hot dist. water, cool and filter. Stain 5-10 min.

"**Kernschwarz H**" is a black water soluble dye used as a nuclear stain for plant material. Make a 4% solution in dist. water and heat it in a boiling water bath for 10 min., cool and filter. Stain 20-30 min. "**Kernschwarz-Kombination H**" is a combination of this black dye with acid fuchsin and picric acid. When prepared like No. 2 above it gives, with animal material, a Van Gieson stain and black nuclei.—*J. A. de Tomasi.*

HURD, C. D. and SCHMERLING, L. Alkenyl derivatives of fluorescein. J. Chem. Soc., 59, No. 1, 1937.

Allyl, pentenyl, and hexenyl derivatives of fluorescein were synthesized. These involved the "ether ester," "diether," "monoether" and "monoester" types. They have very low phenol coefficients.—*Roy L. Mobley.*

PREISLER, P. W. and HEMPELMANN, L. H. Oxidation-reduction potentials of derivatives of thioindigo. I. Thioindigo tetrasulfonate. J. Chem. Soc., 58, No. 11, 1936.

The preparation and tests of thioindigo tetrasulfonate are given. The constant $E_0 = 0.409$ was found for the system and the first pK of the reductant was 4.5.

The substance is suitable for use only where acidity is greater than pH 2.5.—*Roy L. Mobley.*

ROMEIS, B. *Neue Untersuchungen zur Fettfärbung mit Sudan.* *Centbl. allg. Path.*, 66, 97–104. 1936.

Sudan III (Hollborn) of recent manufacture contains chiefly Sudan red, whereas older Sudan III contained considerable Sudan orange and yellow. The new dye is used as follows. Dissolve 1 g. Sudan III in 100 cc. 80% alc. by bringing to boiling in a water bath. Provide the flask with rubber stopper and reflux tube 1 m. long by 6–8 mm. diameter. Let cool to 20° C. then cool further in running water for 30–60 min. Filter next day and store in a tightly stoppered Jena glass flask. This is the stable stock solution. To make the staining solution, filter 20 cc. of stock solution and dilute with 20 cc. dist. water. Put into centrifuge tubes and centrifuge 30 min. Pour off the supernatant fluid, keep well stoppered and filter before use. Stains in 4–5 hr. at 28° or 12–16 hr. at 19–20° C.—*H. A. Davenport.*

T'UNG, T. *Photodynamic action of methylene blue on pneumococcus.* *Proc. Soc. Exp. Biol. and Med.*, 35, 399–400. 1936.

A virulent culture of pneumococcus Type III was grown in Avery's medium for 18 hr. and mixed in a Petri dish with saline saturated with methylene blue in a ratio 9:1. Half was exposed, over a cooling unit, for 45 min. to an electric light of 100 candle power at a distance of 10 cm. The organisms were washed and incubated at 37° C. together with a control suspension preserved with 0.4% formalin. Organisms treated with dye and with light, even after 6 months, were typical in morphology and in reaction to Gram's stain. Smears were treated with alcohol for 2 hr. before staining. Using pneumococcus Type I, dye-treated organisms resulted in better agglutinin titers, and gave better protection in mice than did control suspensions.—*M. S. Marshall.*

ANIMAL MICROTECHNIC

AMPRINO, R. *Un perfezionamento tecnico alla méthode d'Achucarro, pour les fibrilles grillagées, avec quelques considérations sur les méthodes de coloration élective du tissu conjonctif.* *Bull. d'Histol. Appl.*, 13, 223–34. 1936.

This modification of Achucarro's method for reticular tissue, based upon impregnation with Ag, aims to eliminate excessive staining of cells and to sharpen its selectivity for the finer fibrils. The following procedure is recommended: Fix as long as possible in 12% formol or alcohol-formol. Embed in celloidin, cut and affix 6–7 μ sections. Dissolve celloidin and wash in dist. water. Heat slides 30–40 min. in 1–2% tannin in alcohol at 55–58° C. Rinse in dist. water and pass thru 3 jars of Ag-diamine hydroxy-chromate solution, 3 cc.; dist. water, 10 cc. Prepare the Ag solution as follows: To 2 cc. of 20% H_2CrO_4 , add 4–5 more drops of 10% KOH than are necessary to obtain a change of color from reddish to lemon yellow; pour 1 cc. of the chromate solution into 3–4 cc. of 10% $AgNO_3$; wash the precipitate thoroly with dist. water and dissolve in NH_4OH (concentration not specified). Sections must come out of the third jar slightly yellow in color. Rinse 30–40 sec. in dist. water without stirring. Reduce in 30% formol. Each fibril stands out quite clearly against a very light background and artefacts from Ag precipitation should not disturb the preparation.—*J. A. de Tomasi.*

BERBLINGER, W. and BURGDORF. *Neue Färbemethode zur Darstellung der Gewebsbestandteile der Hypophyse des Menschen.* *Endokrinologie*, 15, 381–8. 1935.

The new technic described for the demonstration of the various components of the hypophysis consists of the following steps: Fixation in 4–10% aq. or alc. formalin; hardening and dehydrating in graded alcohols; chloroform; paraffin. Stain sections, after removal of paraffin, 2–24 hr. in an alc. solution of cresofuchsin (0.5–1%); wash briefly in dist. water; then in alum carmine for 8 hr.; rinse in dist. water. Differentiate and stain the acidophil cells for 5 min. in the following solution: 2 g. orange G in 100 ml. of 1% phosphomolybdic acid; rinse in dist. water, place in 5% phosphomolybdic acid for 2 min., dry with blotting

paper, stain for 10–20 min. in: (dist. water, 100 ml.; water soluble anilin blue 0.5 g.; bring to boil, filter cold and dilute filtrate with 2 parts dist. water), rinse in dist. water, differentiate in 75% alcohol until stain no longer washes out. Dehydrate, clear, mount. Chief cells, blue to gray; pregnancy cells, blue with small vivid yellow granules; basophiles with coarse reddish-blue granules; epithelium of the pars intermedia and pars tuberalis, variable; collagen fiber, intensive blue; glia fibers, blue-gray; axons occasionally nearly black. (*Cited from Ber. wiss. Biol.*, 35, 354. 1936. Original not seen; hence the indefinite nature of some of the above dye solutions.)—*J. M. Thuringer*.

BODIAN, D. A new method for staining nerve fibers and nerve endings in mounted paraffin sections. *Anat. Rec.*, 65, 89–97. 1936.

Sharp and selective staining of the nerve elements of the central and the peripheral nervous systems can be accomplished by impregnation of mounted paraffin sections with protargol (silver albumose, Winthrop Chemical Co., New York) and reduction of the silver with hydroquinone. A small amount of metallic Cu must be added to the protargol bath. Mounted sections can be stained in less than 24 hr.

Fixation: With perfusion bottle at height of 6 ft. perfuse rapidly thru the heart with a minimum amount of saline (100 cc. for a cat), followed by 500 cc. of 80% alcohol. Remove central nervous system and place in 95% alcohol for 7–28 days at 37° C. Change alcohol frequently. Perfusion can be started during anaesthesia with chloral hydrate or the barbiturates, or after killing with illuminating gas. If vessels of brain contain much blood, perfusion has not been complete.

Embed in paraffin. Section. Mount. Remove paraffin with xylene and run sections thru abs. alcohol and 95% alcohol to dist. water. Place sections in a solution of 1% protargol containing 4–6 g. metallic Cu per 100 cc. of solution for 12–48 hr. at 37° C. Wash in dist. water. (The protargol solution can be used only once.) Reduce for 10 min. in: hydroquinone, 1 g.; Na_2SO_3 , 5 g.; dist. water, 100 cc. Wash thoroly in dist. water. Tone for 5–10 min. in: AuCl_3 , 1 g.; glacial acetic acid, 3 drops; dist. water, 100 cc. Wash in dist. water. If sections do not have a light purple color, place in 2% oxalic acid until a definite purplish tinge appears (usually 5–10 min.). Wash in dist. water. Place in 5% $\text{Na}_2\text{S}_2\text{O}_3$ for 5–10 min., to remove residual Ag salts. Wash thoroly in dist. water, dehydrate, and mount in balsam. Sections of formalin fixed material should be placed for 24 hr. in a solution of 4% NH_4OH in 95% alcohol before being placed in the protargol solution.—*James B. Rogers*.

COVELL, W. P. A cytologic study of the effects of drugs on the cochlea. *Arch. Otolaryng.*, 23, 633–41. 1936.

This paper deals with the cytological alterations induced in the cochlea by the administration of such toxic agents as quinine and sodium salicylate. In this procedure the step of decalcification is eliminated. Two groups of 16 animals each, including rabbits, guinea pigs and white mice, were given daily for 2–8 weeks, subcutaneous injections of 2.5% quinine bisulfate and 2.5% sodium salicylate, respectively, (200 mg. per Kg. of body weight). A third group was kept as controls. Each animal was anesthetized and perfused with warm saline and with 1:10 formol U. S. P. The histological procedure followed was the one described by Guild (Guild, S. R. War deafness and its prevention: Report on the labyrinths of the animals used in testing of preventive measures. *J. Lab. & Clin. Med.*, 4, 153, 1919). Two series of slides, from each fifth section, of 10 μ are stained with hematoxylin-eosin and, with a modified Van Gieson stain. In the guinea pig it is found that the mitochondrial fixatives, such as Regaud's solution, sufficiently decalcify the thin bone of the cochlea to permit sectioning in paraffin. Proceed as follows: Dissect one cochlea free, fix 3 days in Regaud's, changing daily, transfer to 2.5% $\text{K}_2\text{Cr}_2\text{O}_7$ for 4 days and change daily. Fix the other cochlea 24 hr. in Bensley's A. O. B. liquid. A small opening in the apex of the cochlea and also in the round window permits rapid penetration of fixative. The A. O. B. liquid does not cause distortion or vacuolation in the cells of the stria vascularis, nor of spiral ganglion cells. It is concluded that quinine and salicylate produce drastic alteration and damage to mitochondria and ground substance of cells of those structures.—*J. A. de Tomasi*.

GRIFFITH, J. Q., ROBERTS, E. and JEFFERS, W. A. A staining technic for blood in spinal fluid. *J. Lab. & Clin. Med.*, 21, 1208-10. 1936.

In order to differentiate between a fresh hemorrhage and one more than a day old, the following procedure is suggested:

Dry the spinal fluid smear thoroly for 15-30 min. No fixative is necessary. Immerse 20 min. in 50% alcohol saturated with Sudan III. Rinse 3-4 min. in several changes of dist. water and blot dry. Mount in glycerin. Slides are not permanent as the stain fades in a day or so. Red cells of fresh hemorrhage in cerebrospinal fluid stain uniformly pale yellow; 24-hour old cells show unstained centers and ring-like peripheries.—*J. A. de Tomasi.*

HAUSDORF, G. Über eine histologische und haematologische Schnellfärbemethode. *Deut. Aerzte-Zeitung*, No. 382, 8, 1-4. (Published by DAZ-Verlag Richard Parske, Berlin SO 16.)

A 2% solution of *Nucplascoll* (Hollborn) stains histological preparations and tissue sections within 1 hour. (The name is merely a trade designation and does not indicate the composition of this compounded stain.—*Editor*) Cut pieces of tissue as thin as possible, fix 15 min. in 10% formol at 40-45° C. Rinse in tap water or saline, cut with the freezing microtome. Pass thru water or saline and transfer to a glass containing the staining solution. Heat to vapors (1-2 min.), pass directly into 80% alcohol and finish as usual. Nuclei appear red blue; collagenous connective tissue, brilliant green; muscles and vessels, yellowish-red.

Nucplascoll as blood stain: Fix by flooding slide for 3-4 min. with pure methyl alcohol. Wash in tap water. Stain 30 min. to 2 hr. in 2% *Nucplascoll*, or by heating to light bubbling on a Bunsen flame. Rinse in water and blot dry. Erythrocytes appear green, nuclei of leucocytes, deep blue.—*J. A. de Tomasi.*

HOLLBORN, K. Eine neue Universal-Färbemethode. *Zts. wiss. Mikr.*, 53, 46-7. 1936.

Blockman modified the Van Gieson stain by substituting triphenylrosanilin-sodium trisulfonate (methyl blue?) for acid fuchsin, thus staining the connective tissue blue. This modification, along with chrom-hematoxylin has been embodied into the new universal *Chrom-Hämatoxylin "H"* and *Blockmann-Elastin-Farbstoffe "H"* stain. Dissolve 2 g. chrom-hematoxylin in 100 cc. tap water for 5-10 min. in a boiling water bath. Cool and filter. Stain sections 15 min. Rinse in water and in hartisol or alcohol. Stain 1 hr. in Blockmann-elastin stain under a cover (0.5 g. in 100 cc. of 70% alcohol or hartisol; filter). Rinse with alcohol, clear and mount. *Kernechtrot* can be substituted for chrom-hematoxylin, as a nuclear stain, for 5 min. The results are similar to the Azan stains, with the added advantage of a sharp dark brown contrast coloring for the elastic fibers.—*J. A. de Tomasi.*

JONES, R. L. On the preparation of microscopic sections for making fiber counts of nerves containing unmyelinated fibers. *Anat. Rec.*, 65, 247-54. 1936.

Myelinated and unmyelinated axones of the vagus nerve can be stained in sections made by a combination of the three following procedures. More distinct staining is obtained than by the usual pyridine silver methods. Fixation and silver impregnation follow Ranson's pyridine silver technic (McClung's Microscopical Technique, 2nd ed., p. 349) thru the dehydration step.

1. *Double embedding*: Absolute alcohol 2½ hr., change once; ether-alcohol, 2 hr.; 3½% celloidin, 2-4 days; 7% celloidin, 7-10 days or longer. Place tissue in paper box with 7% celloidin and harden in chloroform vapor 4-6 hr. Trim celloidin on four sides to within 1 mm. of tissue. Roughen two opposite sides of the block. Chloroform, 12-20 hr. Equal parts of chloroform and paraffin (m. p. 52° C.), 24 hr. at 55° C. Paraffin, 12 hr. at 55° C. Paraffin (m. p. 56-58° C.), 6-8 hr. Embed in the same. Mount and trim block so the roughened surfaces of celloidin block are at top and bottom. Cut sections 5 µ thick.

The following method is advised for attaching sections to the slide: Take one end of a strip of lens paper 2 cm. wide and long enough to encircle the slide and attach it to the under surface of the slide with 2% celloidin. Put a few sections on the upper surface of the slide and pull the lens paper tightly around the slide

over the sections and fix the end to the under surface of the slide with celloidin. Keep the slide horizontal while passing thru solutions.

2. *Toning in AuCl₃*: Chloroform, 2 min. to remove paraffin; 95% alcohol, 2 min.; other graded alcohols at intervals of 1 min. down to 50%; dist. water, 1 min.; 0.33% AuCl₃ to which has been added 10 drops of glacial acetic acid for each 50 cc. of solution, 30–50 sec. Remove quickly after sections have changed color. Rinse quickly in dist. water; 5% Na₂S₂O₃, 5 min.; tap water, 5 min.; dist. water, 1 min.; dehydrate by graded alcohols, at intervals of 1 min. up to 95% alcohol. If counterstain is not desired, dip for a few seconds in abs. alcohol; clear in oil of thyme and clove oil, 5:1. Mount as described later.

3. *Counterstaining with azocarmine*: Tone in AuCl₃ and dehydrate as described above. Mordant in anilin alcohol (1 cc. anilin oil in 1000 cc. of 95% alcohol), 15 min.; azocarmine (Grübler's) 1 g.; dist. water, 100 cc.; glacial acetic acid, 1 cc., in closed dish at 56° C., 15 min. Rinse in dist. water, removing excess only. Pass quickly thru graded alcohols, differentiate in anilin alcohol until cytoplasm becomes pinkish; 1 min. is usually sufficient. Grossly, sections remain a deep red. Acetic alcohol (1 cc. glacial acetic acid in 1000 cc. 95% alcohol), 1 min. to wash out anilin alcohol; 5% aq. phosphotungstic acid, 15 min.; 30 min. in the following stock solution diluted with equal parts of water: anilin blue, W. S., 0.5 g.; orange G, 2.0 g.; glacial acetic acid, 5.0 cc.; dist. water, 100 cc., dissolved by heating till it steams for 7 min.; filter after cooling. Rinse in dist. water, pass quickly thru graded alcohols (4–5 sec. in each), differentiate in 95% alcohol. Watch sections and remove when the nerve, very dark at first, becomes moderately blue. Pass quickly thru abs. alcohol, clear in oil of thyme and clove oil, 5:1; mount as follows: Drain the excess of clearing fluid; remove lens paper; blot on towel, section side down; straighten and remove wrinkles with camel's hair brush wet in clearing fluid. A small amount of thick balsam is spread on a cover slip and the cover slip is placed over the sections. Put a square piece of glass on the cover slip and hold it in place with a strong spring paper clip. The square of glass may be cut from a slide and should be slightly smaller than the cover slip. Dry slides at 40–50° C. for 1 or 2 days.

Results: Celloidin, by preserving the orientation of fibers, helps in the identification of closely packed axones. Toning in AuCl₃ practically clears the background of Ag-stained sections, while the unmyelinated axones appear black. Azocarmine does not stain the axis cylinders, but myelin sheaths stain orange, the nuclei of Schwann cells, light red, and the background, light blue.—James B. Rogers.

KINOSITA, R. and DAIKOKU, K. A new fixative for tissue glycogen and the features of liver glycogen when it is applied. *Trans. Jap. Path. Soc.*, 26, 127–30. 1936.

Experiments in search of a fixative suitable in staining methods for tissue glycogen show that 95% alcohol alone, as well as saturated solutions of KCl or MgSO₄ in 95%, 80% and 50% alcohol are all equally good. The fixing properties of 4% formaldehyde, saturated with KCl or MgSO₄, are as good as those of diluted alcohol containing these chemicals. These methods are an advantage because they permit use of paraffin or celloidin which give thinner sections. KCl gives different results from MgSO₄. With the former, the boundary of each liver cell is distinct. The glycogen granules are finely divided and stain more brightly. They are aggregated more densely near the cell membrane and around the small vacuoles while they are more or less evenly distributed thruout the protoplasm. With MgSO₄ on the other hand, the boundary of each liver cell is indistinct and the glycogen occurs in the form of larger, irregularly grouped granules which are closely aligned around the small vacuoles of the cell. Formaldehyde fixation gives finer granules.

In another modification, a stronger solution of formaldehyde is used. When fairly large portions of tissue are to be fixed it is best to use 40% formaldehyde containing isotonic KCl.—Elizabeth Bachelis.

KONEFF, A. A. An iron-hematoxylin-anilin-blue staining method for routine laboratory use. *Anat. Rec.*, 66, 173–9. 1936.

This method stains simultaneously most histological elements in paraffin, celloidin, or frozen sections after common fixatives, including Zenker and Zenker-for-

mol (which gives best results). Mordant sections in 5% aq. solution of $\text{Fe}_2(\text{SO}_4)_3 \cdot (\text{NH}_4)_2\text{SO}_4 + 24 \text{ H}_2\text{O}$ for 5-10 min. Rinse quickly in dist. water. Stain in Harris' hematoxylin for 3-15 min. (Good results have also been obtained with 0.5 to 1.0% alc. hematoxylin, freshly prepared or ripened; with 1% aq. hematoxylin prepared by boiling for 5-10 min.; and with Delafield's hematoxylin. The staining time must be determined for each type of hematoxylin.) Wash in dist. water. Place in the following mixture, which keeps well: anilin blue (Grübner), 0.1 g.; oxalic acid, 2.0 g.; phosphomolybdic acid, 15.0 g.; dist. water, 300 cc. Wash in dist. water, differentiate and dehydrate in 2 changes of abs. alcohol, clear in xylene, and mount in balsam. If euparal is used as the mounting medium, the xylene may be omitted. When staining loose celloidin sections, substitute 95% alc. for abs. alcohol and mount in euparal. Clearing in creosote is not necessary unless the sections are mounted in balsam.

The staining results are: nuclei, violet brown; cytoplasm, light brown; erythrocytes, dark violet; myelin and muscle fibers, brown; elastic fibers, reddish brown or red. This stain can be used in connection with reticulum impregnation and gives very sharp outlines.—S. I. Kornhauser.

LINDAHL, E. *Methode de décalcification des objets riches en carbonate de calcium.* *Bull. d'Histol. Appl.*, 12, 216-9. 1935.

The author decalcifies delicate zoological objects (sponges, echinoderms, etc.) by converting their CaCO_3 into $\text{Ca}(\text{HCO}_3)_2$ (water soluble) by passing CO_2 thru an aqueous suspension either at atmospheric pressure or under a pressure of 3-4 atmospheres. A special apparatus is described. (*Cited from Ber. wiss. Biol.*, 34, 259. 1935.)—J. M. Thuringer.

MALLORY, F. B. *A lead hematoxylin stain for axis cylinders.* *Amer. J. Path.*, 12, 569-71. 1936.

Fix 3 mm. tissue sections in 10% neutral formalin (time not specified). Mordant 6 weeks at room temp. or 7 days at 37° C. in sat. aq. solution of PbCl_2 . Change fluid at end of 24 hr. and once or twice later. Wash 24 hr. in running water. Preserve in 80% alcohol. Embed in celloidin or paraffin. *Staining solution:* Dissolve 1-5 mg. hematoxylin in 1 cc. 95% alcohol and add 10 cc. sat. solution of CaCO_3 in dist. water. Coarse nerve fibers stain in 30-60 min. at room temp., fine fibers 3 hr. at 54° C. For a more differential stain, put unstained sections 1 min. into a solution of: I_2 , 0.1 g.; KI , 0.2 g.; dist. water, 100 cc. Wash in water and 2 or 3 changes of 95% alcohol to extract iodine (do not use $\text{Na}_2\text{S}_2\text{O}_5$). Wash in water and stain as above. Dehydrate, clear, and mount.—H. A. Davenport.

MARTIN, J. F. and DELAUNAY, A. *Quelques points de technique pour les préparations histologiques du tissu osseux.* *Bull. d'Histol. Appl.*, 13, 457-9. 1936.

Several suggestions are offered for improving the histological treatment of bony tissues. By holding a piece of bone gently between two cork discs in a vice, cut plates 1-2 cm. thick with a surgical saw. Fix (at least 2 weeks) in saline-formol, Bouin's, Helly's, or sublimate-formol-acetic liquid. Where acetic acid is specified the same volume of 5% trichloroacetic acid will give less hemolysis. Fix 24-48 hr. in a vacuum as high as 70 mm. Wash at least 24 hr. in running water. Decalcify with 1% trichloroacetic acid in the same degree of vacuum, changing once a day for about 8 days. Wash 24 hr. in running water until neutral to litmus. Dehydrate 48 hr. in abs. alcohol, changing twice. Embed by either of the two alternate procedures. (1) Soak 12 hr. in each of the following: 1:1 amyl acetate and abs. alcohol; amyl acetate; 1:1 amyl acetate and paraffin (52-54° C.); 3 changes of paraffin at 57° C. (2) After dehydration transfer to: methyl benzoate, 500 g.; celloidin, 5 g. In about 4 hr. the specimen will sink to the bottom. During the following 24 hr. change 3 times in the same liquid. Soak 24 hr. in benzine, changing once; transfer to 1:1 benzine and paraffin in an oven at 40° C. Follow 5 hr. in paraffin at 57° C., changing twice. Cool and block.

This procedure gives usual bone staining and preserves even the cytological details of the medullary tissue.—J. A. de Tomasi.

PRENANT, M. Emploi du nitrate d'argent pour l'étude de la texture osseuse. *Compt. Rend. Soc. Biol.*, 123, 472-3. 1936.

Two modifications of the von Kossa silver method are proposed for better demonstration of the fine structure of compact bone: (1) Preliminary soaking of the bone in 0.04-0.09% agar to make diffusion of AgNO_3 uniform. (2) Impregnation with lower concentrations of AgNO_3 (0.5-0.005%).—*H. E. Jordan.*

SCHABADASCH, A. Histophysiologie des réactions réciproques entre le bleu de méthylène et le tissu nerveux. III. Influence des ions sur la coloration. Signification particulière des ions-Mg. Conclusions générales. *Bull. d'Histol. Appl.*, 13, 137-51. 1936.

The conditions regulating vital staining of nervous tissue by methylene blue are discussed. The Mg ions in the following solution have a favorable effect: NaCl, 8 g.; glucose, 2 g.; sodium pyruvate, 0.324 g.; MgBr_2 , 1.5 g.; methylene blue, 0.25 g.; dist. water, 1000 cc. Adjust to pH 6 with phosphate buffer. Injection of 1500 cc. into a cat gives a satisfactory specific staining of the peripheral and cerebrospinal nervous systems.

Conclusions: Methylene blue is an energetic receptor of H^+ . The presence of H ions causes substantial changes within the tissues and represents a necessary step toward the final reaction between the dye and the colloidal structures of nervous tissue. The presence of glucose or certain products of its metabolism (e. g., pyruvate) is also necessary. The addition to the dye of H receptors of the quinone group (resorcin and pyrocatechin) provides new methods for staining nervous tissue.—*J. A. de Tomasi.*

SEKI, M. Zur Kenntnis der intra- und supravitalen Färbung. VII. Färbbarkeit der Blutmonocyten und ihre elektrische Ladung. *Zts. Zellforsch. Mikr. Anat.*, 22, 707-19. 1935.

The author concerns himself with the question of acidophilia and basophilia of the monocytes in comparison with other blood cells in various mammals. Stains employed: Pappenheim's panoptic stain, eosinate of methylene blue after methyl alcohol fixation, and methyl alcohol fixation followed by treatment with picric acid. All staining solutions were buffered to pH 7.0. The basophilia of the intergranular plasma substance measured by the intensity of the stain ranged from lymphocytes, monocytes, eosinophiles to special leukocytes. After treatment with picric acid which precipitated basic substances and removed the acid substances, it was found that the following serial arrangement indicated the acidophilic character of the plasma substance: monocytes, lymphocytes, special leukocytes, and eosinophiles. No appreciable differences in staining were noticed regardless of the animal from which the blood was obtained. The monocytes are very rich in acidophilic as well as basophilic substances, hence of a pronounced amphoteric or polyphase character. The acidophilic constituents of the cytoplasm colloids appear to play a significant role in vital staining with collargol, India ink and trypan blue. (Cited from *Ber. wiss. Biol.*, 34, 516. 1935.)—*J. M. Thuringer.*

STEWART, R. J. C. A short Marchi technique. *J. Path. & Bact.*, 43, 339-43. 1936.

The Marchi technic, for degeneration of myelinated fibers, is open to several objections: Müller fixed material cannot be used with all stains; long storage in the fixative prevents satisfactory staining of the degenerated myelin; it is a lengthy procedure; and artefacts are fairly common. The following shortened Marchi schedule requires only 2 days (exclusive of fixation), overcomes most disadvantages of the original technic, and has been found satisfactory in over 3 years of routine application:

Fix pieces 2-3 mm. thick at least 2 days (preferably 8-10) in 10% neutralized formol-saline. Change after first 24 hr. Cut 30 μ frozen sections. Wash 1½ hr. in many changes of water, using dist. water for the last 4 washings. Place in 2.5% $\text{K}_2\text{Cr}_2\text{O}_7$ for 24 hr. at 21° C. Wash in dish of dist. water until almost all the yellow color disappears. Transfer to 1% osmic acid in the dark for 16-36 hr. The degree of blackening can be judged only by experience. The following treatment may be used if normal myelin is too dark. Wash 5 min. in tap water; treat 30 sec. with 0.05% KMnO_4 ; rinse with tap water; and hold in Pal's solution

(1% oxalic acid and 1% K_2SO_4 , 1:1) until all brown deposit is removed. Wash in many changes of tap water. Pass thru 70% alcohol, 1-2 min.; 90% alcohol, 1-2 min.; abs. alcohol, 1-2 min. Mount in Gurr's neutral medium; harden 24 hr.; remove excess medium and ring with Krönig's Deckglaskitt (add gradually 8 parts colophonium resin to 2 parts melted paraffin wax, m. p. 54° C., mix well, cool).

The products of degenerated myelin are stained black. Alternative methods employing Scharlach R and methylene blue give less satisfactory results.—*J. A. de Tomasi.*

SZANTROCH, Z. Beitrag zur Fettfärbung in den Gewebekulturen (Deckgläschenkulturen) und histologischen Schnitten mit Sudanlösung in Alkoholformol. *Arch. exp. Zellforschung*, 17, 206-9. 1935.

For simultaneous fixation and staining the author formerly proceeded as follows: Sat. solution of Sudan (no further information available) in 95% alcohol, 4 parts; 40% formalin, 1 part. Mix just before using. Stain from ½-5 min. Disadvantages of above method are: poor keeping qualities of preparations; confluence of fat droplets; and precipitates in culture media. The author now suggests the following improved method: 95% alcohol, 90 cc.; 40% formalin, 30 cc.; glacial acetic acid, 2 cc.; Sudan in excess. Let stand at room temp. for at least 2 weeks. Float preparation on stain from ½-5 min. Wash carefully. Counterstain in Delafield's hematoxylin. Mount in glycerin, seal cover glass with wax. For frozen sections, fix 24 hr. in 5% $K_2Cr_2O_7$, 8 parts; 40% formalin, 2 parts. Wash in running water for 24 hr. Stain 5-15 sec. (Cited from *Ber. wiss. Biol.*, 34. 1935.)—*J. M. Thuringer.*

PLANT MICROTECHNIC

CARLSON, J. G. Effects of several fixatives on staining reactions in *Zea mays*, especially with reference to the Feulgen reaction. *Cytologia*, 7, 104-17. 1936.

The Feulgen stain is specific for chromatin, while hematoxylin can be made specific for any of the cell parts, depending on the fixative employed. This study is concerned with the effects of fixatives on staining. Root tips of *Zea mays* are fixed 40-48 hr. in the following fixatives: Bouin's, Navashin's, $CuCr_2O_7$, copper-chrome-propionate, nickel-chrome-propionate, formol-sulfuric acid, formic acid-acetaldehyde, modified Erlicki, and chromic sulfate-formaldehyde. Tissues are then washed 1 hr. in tap water, dehydrated, cleared, infiltrated and embedded by the butyl alcohol method. Slides are divided in two series, one of which is hydrolyzed for 20 min. at 60° C. in *N HCl*. Sections are stained with the Feulgen stain, Heidenhain's iron-alum-hematoxylin, crystal violet iodine, and safranin iodine (2% for 10 min.).

Results: The Feulgen reaction is chromatin-specific. The cuticle is also stained, showing a definite fixation effect because the color intensity varies with the nature of the fixative while specificity depended upon the type of chromatin stain. Fixation influences the staining reactions of different cell structures produced by hematoxylin, crystal violet and safranin. Crystal violet and safranin show consistent uniform selectivity. The *HCl* treatment affects the relative staining capacity of plastin and chromatin by hematoxylin, crystal violet and safranin, after some of the fixatives. *HCl* treatment after Bouin's and Navashin's fixatives lowers the stainability of chromatin by hematoxylin but improves the staining of nucleoli after Navashin's fixative. The effect of the *HCl* treatment upon the staining of chromatin or nucleoli by crystal violet and safranin varies definitely with the kind of fixative used.—*J. A. de Tomasi.*

HUSKINS, C. L. On the cytology of speltoid wheats in relation to their origin and genetic behavior. *J. Genetics*, 20, 103-22. 1928.

Preliminary cytological fixations were made from greenhouse plants with Allen's Bouin, Carnoy (6:3:1 mixture), Flemming, and Kihara (Mem. Coll. Sci., Kyoto Imp. Univ., Ser. B, 1, 1-200. 1924) fixatives, and various modifications of the latter. Allen's Bouin gave the most uniform results, and a high general standard of fixation. Kihara's method, while somewhat variable, gave results

which were in most cases superior to any of the others. This method consists in fixing for 1 or 2 min. in Carnoy and then for 24 hr. in Flemming. In a modification of it which gave similar results, Zenker fluid with only 1 or 2% acetic acid was substituted for Flemming. This obviated the necessity of bleaching. Fixations of field-grown material were made principally by the Carnoy-Flemming or Carnoy-Zenker method. The anthers were dissected out in some cases, but fixations were chiefly made of spikelets with the ends of the glumes clipped off.

Newton's iodine-gentian-violet staining method (C. L. Huskins, *J. Genetics*, 18, 315-64. 1927) was used almost exclusively, and gave results far superior to any other. It gave a particularly brilliant stain after the Carnoy-Zenker fixative.

All material was embedded in paraffin and cut, usually at 14 μ . The study was almost entirely confined to the pollen mother-cells, but a few examinations have been made of embryo-sac mother-cells and of root tips, the latter fixed in medium Flemming and Benda.—*R. W. Cumley.*

LUYET, B. J. Differential staining for living and dead cells. *Science*, 85, 106. 1937.

Good results on plant material are secured with the following procedure: Lay on a slide, cutin side down, a portion of the lower epidermis of onion bulb. Cover 2 min. with a drop of 0.5% slightly alkaline neutral red solution. Blot and replace by 1 drop of 0.4% KOH. Blot immediately and wash with tap water. Living cells take a bright cherry red, dead cells are intense orange yellow. KOH is injurious in concentrations above 1%, but is not instantly lethal up to 2.5%.—*J. A. de Tomasi.*

MILOVIDOV, P. F. Zur Theorie und Technik der Nuklealfärbung. *Protoplasma*, 25, 570-97. 1936.

The claims of several investigators that Feulgen's reaction does not occur in the cell nuclei of many plants are denied by the author who interprets the negative finds of others as the result of incomplete technic and, in many cases, may be due to the presence of tannates in the cell.—*R. Chambers.*

OURA, G. A new method of unravelling the chromonema spirals. *Zts. wiss. Mikr.*, 53, 36-37. 1936.

Meiotic chromosomes in pollen mother-cells of *Tradescantia reflexa* are ordered in double coiled chromonema spirals. They can be unravelled by the following procedure. Spread out the cells on a slide, and add some aq. KCN (2- to 2-4 mol.) for a few minutes. Blot dry and stain with aceto-carmine. Heat the slide over the flame of a match. Other weak alkaline salts that can be used are NaOCl and NaHCO₃.—*J. A. de Tomasi.*

PHILP, J. and HUSKINS, C. L. The cytology of *Matthiola incana* R. Br. especially in relation to the inheritance of double flowers. *J. Genetics*, 24, 359-404. 1931.

Root tips were taken from the seedlings and fixed in most of the standard fixatives used for plant cytology. Benda's fluid gave the best results. Ovules could not be fixed satisfactorily in any of the standard fluids tried, but good results were obtained from La Cour's first fixative (*Nature*, 124, 127. 1920). Pollen mother-cell smears made according to Taylor's method (*Bot. Gaz.*, 78, 236-8. 1924) were tried, and a number of fixatives, including La Cour's and modifications of it, again gave the most satisfactory results. Satisfactory fixations of pollen mother-cells were also obtained by aceto-carmine methods. In making permanent aceto-carmine mounts prior fixation in acetic alcohol proved undesirable.—*R. W. Cumley.*

MICROORGANISMS

CHAPMAN, G. H. Specificity of the dye in the crystal violet agar reaction of staphylococci. *J. Bact.*, 32, 199-205. 1936.

Staphylococci produce orange, violet or white growths on crystal violet agar. An attempt is made to find some explanation for these variable reactions, and to identify the active molecule of the dye. In order to determine the active chemical group, 10 strains of staphylococci are tested against 108 dyes, covering all the

range of biological dyes. Dilutions, in the agar base, of 1:10,000, 1:100,000, 1:250,000 and 1:500,000 are compared, in terms of growth color, with crystal violet (dilution 1:300,000 to 1:350,000). Significant color changes are only noted in the case of 5 samples of methyl violet, 1 of crystal violet, and 1 of Hofmann violet. Comparable results with brom thymol blue are attributed to its indicator properties.

A closer study of the violet phenylmethanes covers 60 samples including those labeled dahlia, dahlia B, crystal violet, methyl violet, gentian violet, ethyl violet, Hofmann violet. Samples of methylene violet were tested also. It appears that the only dyes capable of giving the color reaction are those suspected of containing methylated pararosanilins, particularly the higher homologs (penta- or hexamethyl pararosanilin). For this reason and because of its uniformity, Commission certified crystal violet is to be preferred for the preparation of crystal violet agar. Solutions of the dye may lose specificity on standing.—*J. A. de Tomasi.*

DIENST, R. B., and SANDERSON, E. S. Use of nigrosin to demonstrate *Treponema pallidum* in syphilitic lesions. *Amer. J. Pub. Health*, 26, 910. 1936.

The characteristic morphology of this organism can best be demonstrated by darkfield illumination or by methods which stain the background, leaving the organism colorless. For the latter purpose the authors find nigrosin far superior to other agents. Successful results depend upon the selection of the most suitable material for examination and the most suitable method of collection. Exudate should be obtained by squeezing cleaned and dried primary ulcers or papules. Glandular fluid should be obtained with a sterile syringe.

Wet preparation: Place a small loopful of the exudate on a clean slide and add a loopful of 5% aq. nigrosin to which 0.5% formalin has been added. Mix thoroly and spread over slide so that part of smear is thin. Pass slide over flame to dry rapidly. Place drop of immersion oil directly on smear and examine.

Dry preparation: Allow a drop of exudate to air dry on a slide. This may be sent to the laboratory and stained even as much as 45 days later, altho the sooner the better. To stain, place a small loopful of water on the dried exudate, allow to diffuse for a few seconds, then apply a loopful of the nigrosin solution, smear, dry, and examine as before.

This method checked well with Wassermann, Kahn, and darkfield reactions, and is proposed to replace darkfield examinations in state laboratories.—*M. W. Jennison.*

GELARIE, A. J. A new, one-minute method for the staining of spirochetes, spirilla, spermatozoa, and related organisms. *J. Lab. & Clin. Med.*, 21, 1065-9. 1936.

Shortcomings of Burri's, Levaditi's, and Giemsa's methods for the examination of fluids containing spirochetes and spirilla are pointed out. Directions for a quick method are: Smear the material thinly and air-dry. Fix 5 sec. in a zirconyl chloride solution (2.5 g. recrystallized $ZrOCl_2$ dissolved in 100 cc. 10% aq. $NaCl$). Wash with water. Treat 10 sec. with a few drops of 10% aq. recrystallized citric acid solution (preserve with thymol). Wash with water. Stain 30 sec. with 0.25% gentian violet in $N/10$ sodium lactate (approx. pH 8.4). Wash with water. Cover a few seconds with K_2HgI_4 solution (dissolve 0.5 g. HgI_2 in 100 cc. 0.5% aq. KI). Wash with water. Counterstain 5 sec. with carbol methylene blue (dissolve 0.2 g. methylene blue in 100 cc. dist. water, add 0.2 g. phenol). Wash and blot dry. Red blood cells show finely granular rose colored cytoplasm; chromatin of leucocytes, blue; spirilla, deep purple. Mechanism of the stain is explained on a chemico-physical basis, assuming differences in the isoelectric points of constituents of organisms and those of red blood cells. Substitution of Al, Bi, Cd, Mg, Hg, Th, U, Cu and Sn chlorides does not give satisfactory results.—*J. A. de Tomasi.*

MALAKOFF, M. T. A technique for the slide culture of fungi. *Science*, 84, 490. 1936.

A method is given for setting up slide cultures of various fungi which are also suitable for permanent mounting. Sterilize the slide in individual 30 x 140 mm. test tubes. Melt sterile agar medium, tubed in 3-5 cc. quantities, and cool to

point of gelation. Pour aseptically over the slide. This affords hardening of medium in an uneven layer which upon inoculation permits all stages of growth. In 48 hr. at room temperature slides are dry enough for mounting. Fix in: 50% alcohol, 100 cc.; formalin, 6.5 cc.; glacial acetic acid, 2.5 cc. Pass thru 35% alcohol and dist. water. Stain 5 min. in an acid dye, wash thoroly in dist. water, dry overnight and mount.—*J. A. de Tomasi.*

TSUCHIYA, H. The effects of dyes on *Endameba histolytica* in vitro. *J. Lab. & Clin. Med.*, 21, 1028-35. 1936.

A study is reported of the viability of cysts and trophozoites of *E. histolytica* when exposed to the action of various dyes, with a view to determining any changes of cell structure or any dye resistance developed. For the viability of cysts, eosin proves to be quite inadequate. Other dyes used are: gentian violet, malachite green, brilliant green, pyoktanin, acid and basic fuchsin, acriflavine, neutral red, methylene blue, safranin, brilliant cresol blue, toluidine blue, Bismarck brown, eosin B and mercurochrome. To 5 cc. of dye dilution, 0.1 cc. of washed cysts is added. After incubation at various temperatures and at varying time intervals, 0.1 cc. is diluted in 0.9 cc. nutrient broth, and 0.1 cc. of the dilution is inoculated into the author's S. C. culture medium with Dorsett's egg slant (Tsuchiya, H. Further studies on the cultivation of *Endameba histolytica* and a complement fixation test for amebiasis. *J. Lab. & Clin. Med.*, 19, 495. 1934).

Upon incubation at 37° C. the culture is examined in fresh preparation, or stained with iron hematoxylin, Tsuchiya's technic (Tsuchiya, H. A practical staining method for intestinal protozoa. *J. Lab. & Clin. Med.*, 17, 1163. 1932) or Gram's technic. To study the effect of dyes on the trophozoites, 0.5 cc. of each dilution of dye is added to 4.5 cc. of a growing culture, taking observations at 24, 48, and 72 hr. at 37° C. Surviving cultures are transferred to fresh culture medium and exposed to equal dye concentrations. High concentrations of the dye have an amebicidal action on cysts. Dilution results in amebastasis, displayed best by gentian violet, acriflavine, malachite green, and to a lesser extent by basic and acid fuchsins. Amebastasis is accompanied by an increased size of the organisms. Action on trophozoites is usually amebicidal. Resistance to gentian violet, acid fuchsin and malachite green can be built up.—*J. A. de Tomasi.*

HISTOCHEMISTRY

FAUTREZ, J. Sur le point isoélectrique des cellules du système réticulo-endothélial des vertébrés. *Bull. d'Histol. Appl.*, 13, 202-6. 1936.

The isoelectric point of the cytoplasm of reticulo-endothelial cells is found to lie in the same pH region as in the case of other cells. This point has been investigated by staining sections of the tissue 10 min. with cyanol, an acid dye, and toluidine blue, a basic dye, at various pH. Results do not agree with Seki's findings (Seki, M. *Zts. Zellforsch.*, 19, 238. 1933).—*J. A. de Tomasi.*

GÖMÖRI, G. Microtechnical demonstration of iron. *Amer. J. Path.*, 12, 655-63. 1936.

Formalin and formalin-alcohol (no formulae specified) fixed organs containing iron bearing pigments were subjected to standard staining methods for iron. No traces of ferrous compounds were found by the direct Turnbull's blue reaction. The best microtechnical reagent for Fe was found to be a mixture of equal parts of 1:4 HCl and 10% $K_4Fe(CN)_6$. Sections should be soaked 30 min. Results of similar quality were obtained by treating with $(NH_4)_2S$ (Quincke's reagent) 1 hr., washing with dilute NH_4OH , dist. water, and finally 1 hr. in 5% $CuSO_4$ or $Pb(NO_3)_2$. Fading of Berlin blue and Turnbull's blue preparations was prevented by thinning nearly dry Canada balsam with oxidized oil of turpentine.—*H. A. Davenport.*

LUCAS, M. S. and EVANS, C. A. Correlation of qualitative microchemical tests on the protozoan nucleus and the mode of nutrition. *J. Royal Microsc. Soc.*, 55, 261-4. 1935.

This paper is a discussion of the relationship between the chromatin of the nucleus in certain protozoa, as revealed by the Feulgen technic, and the mineral

constituents of it, as shown by microincineration. The following observations are made: the protozoan nucleus does not necessarily contain its nucleic acid in the form of thymonucleic acid. There are instances where chromatin in the fixed nucleus stains with basophilic dyes, but reacts negatively with the Feulgen test. This cannot, therefore, be considered a universal test for chromatin. Furthermore, the nucleus in saprozoic protozoa is deficient in inorganic salts which will withstand incineration. These two main points cast doubt on the reliability of comparisons between protozoan and metazoan nuclei.—*J. A. de Tomasi.*

MARZA, V. D. and CHIOSA, L. T. **Histochimie quantitative du potassium dans les ovules en croissance.** *Bull. d'Histol. Appl.*, 13, 153-77. 1936.

The role of K in biological processes is known to be a very important one. This extensive paper deals with the part played by this element in cellular metabolism as determined by studying the quantitative histochemical variations in hen's ova. Twelve ovaries are fixed in 96% alcohol, dehydrated in abs. alcohol, cleared, embedded in paraffin and cut 7μ thick. Determination of K is carried out by means of the microcomparator (Marza, V. D. and Chiosa, L. *Essai de detection histochimique quantitative du potassium.* *Bull. d'histol. Appl.*, 12, 58-72. 1935).

Results: K displays slight tendency to concentrate in ovular membranes. Its accumulation in the ovum is in direct relation to the appearance of the vitellus.—*J. A. de Tomasi.*

URAKAMI, T. **Ein neues Verständniss des Unnaschen Sauerstoffortes und Reduktionsortes.** *Trans. Soc. Path. Jap.*, 24, 96-103. 1934.

Histological staining is based upon an adsorption phenomenon between 2 colloids having opposite charges, i. e., the tissue protein and the dye. The least staining, therefore, occurs at the iso-electric point of the tissue protein and conversely the disappearance of the staining may be used as an indicator of the i. e. p. at a given pH.

Technic: Fresh formalin, or alcohol fixed sections are stained with methylene blue or crystal ponceau solutions, buffered in steps from pH 2 to 7 and washed with corresponding buffer solutions, treated with 4% $(\text{NH}_4)_2\text{MoO}_4$, alcohol, xylene, balsam. The results are presented in a table. Ordinarily the i. e. p. of the nucleus is more on the acid side than the cytoplasm. Hardening with formalin produces a shift of the i. e. p. toward the acid side, with alcohol toward the alkaline side. The author rejects Unna's theory of oxidation and reduction regions in cells, assuming that the differences in staining with the rongalit white technic were not brought about thru variations in oxidation or reduction intensity but thru differences in pH which changed the electrical charge and with it the staining qualities. (*Cited from Ber. wiss. Biol.*, 34, 516. 1935.)—*J. M. Thuringer.*

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the January number of this Journal.

STAINS CERTIFIED DECEMBER 1, 1936 TO FEBRUARY 23, 1937*

Name of dye	Certification No. of batch	Dye Content	Object of tests made by commission†	Date approved
Alizarin red S	NAr 1	—	For staining bone	Dec. 4, 1936
Alizarin red S	LAr 1	—	For staining bone	Dec. 4, 1936
Crystal violet	CC 8	88%	As histological, cytological and bacteriological stain	Dec. 19, 1936
Fast green FCF	NGf 3	93%	As histological counter-stain	Jan. 4, 1937
Pyronin G	NP 6	58%	As constituent of Pappenheim stain	Jan. 12, 1937
Tetrachrome stain	NMn 9	—	As blood stain	Jan. 23, 1937
Cresyl violet	NW 6	92%	For use in histology	Feb. 25, 1937

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

STAIN TECHNOLOGY

VOLUME 12

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PROGRESS IN THE STANDARDIZATION OF STAINS¹

DYES FOR USE IN BACTERIOLOGICAL MEDIA

There are several dyes which are used in bacteriological media primarily because of their selective bacteriostatic powers. The dyes ordinarily employed are members of the tri-phenyl methane group. The question of standardizing dyes for such purposes has been frequently discussed²; and at present this standardization seems to have been pretty well effected in the case of three dyes, namely, crystal violet, basic fuchsin and brilliant green.

These three dyes are called for in certain media specified in the various Standard Methods reports issued by the American Public Health Association. In the latest editions of these reports it is insisted that dyes should be used for these purposes only when they have been approved by the Commission on Standardization of Biological Stains for use in bacteriological media. This requirement on the part of the American Public Health Association has made it necessary for the Stain Commission to test all samples of these three dyes submitted for certification to see whether they are satisfactory as bacteriostatic agents.

The present notice is really to call attention to the fact that this procedure is now in routine use. For something over a year all samples of these three dyes submitted have been tested for this purpose, and if satisfactory they are allowed to be sold bearing the Commission certification with a statement on the main label of the dye reading "For use in bacteriological media". Those who wish to use dyes for this purpose are advised to look for this statement on the label as well as the certification statement of the Commission. With some stain manufacturers both statements are incorporated on the same label, with others they appear on separate labels.—H. J. CONN.

¹Notes under this heading have appeared in earlier numbers of STAIN TECHNOLOGY, the last of the series having appeared July, 1935.

²See Conn, H. J. The bacteriostatic use of dyes. Stain Techn., 10, 1. 1935.

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since the last one listed in the April number of this journal.

STAINS CERTIFIED MARCH 1, 1937 TO MAY 31, 1937*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Acid fuchsin	CR 6	58%	As histological stain, and for use in Andrade indicator	Mar. 5, 1937
Sudan IV	NZ 9	86%	As fat stain	Mar. 15, 1937
Giemsa stain	BGe 1	—	As blood stain	Mar. 16, 1937
Brilliant cresyl blue	NV 15	62%	For vital staining of blood	Mar. 24, 1937
Giemsa stain	GGe 4	—	As blood stain	Mar. 30, 1937
Hematoxylin	FH 13	—	As histological and cytological stain	Apr. 8, 1937
Methylene blue	NA 12	87%	For histology, bacteriology, and as constituent of blood stain	Apr. 20, 1937
Basic fuchsin	NF 29	94%	For general staining, the Feulgen reaction, and in Endo medium	Apr. 22, 1937
Nigrosin	NNi 6	—	For negative staining of bacteria	May 16, 1937
Anilin blue WS	CK 4	—	As histological stain	May 11, 1937
Methylene blue tablets	NAt 1	92%	For reductase test of milk	May 13, 1937
Eosin Y	LE 11	93%	In histology and as constituent of blood stain	May 24, 1937

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

SPLIT NUCLEOLI AS A SOURCE OF ERROR IN NERVE CELL COUNTS

RUSSELL L. JONES, *Indiana University, School of Medicine,
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ABSTRACT.—The number of nerve cells in a given ganglion or nucleus is usually determined by counting the nucleoli in serial sections. The possibility that nucleoli may split and appear in more than one section is recognized as a source of error. A determination of the value of this error was made as follows; from nodose ganglia of four cats were cut serial transverse sections in which the sections varied in thickness. Thus from one ganglion, four sections were cut at $12\ \mu$, then six at $9\ \mu$, and eight at $6\ \mu$. The process was repeated over and over until the entire ganglion was sectioned. The other ganglia were sectioned similarly. After mounting and staining, separate counts were made of the nucleoli of each given ganglion from the sections of different thicknesses. If nucleoli split according to theoretical expectations, the percentage of nucleoli split in thick sections should be less than the percentage split in thinner sections and the counts based on the sections of different thicknesses should vary accordingly. The results obtained indicate that the counts from thin sections do not differ appreciably from counts from much thicker sections, i. e., the thickness of the sections does not affect the count. It is, therefore, concluded that no correction should be made for split nucleoli if the sections are around $10\ \mu$ in thickness and none but distinct and definite nucleoli are counted.

The number of nerve cells in a given ganglion or nucleus is usually determined by counting the nucleoli in serial sections. One common method is to count in serial sections $10\ \mu$ thick the nucleoli in the first three consecutive sections of every fifteen, arriving at the total number by a calculation based on the sections counted. Three adjacent sections are counted to minimize any error due to the variation in the number of nucleoli in adjacent sections.

In addition to the sources of error incident to any method involving the counting of microscopic structures, there is in the above method of cell counting a source of error resulting from split nucleoli which ap-

pears in each of two sections. This source of error has been recognized but investigators differ in their estimates of its percentage value. Davenport and Ranson (1931) report that of several hundred cells examined in serial sections, only 5% of the nucleoli were split and that one-half of those split were stained so poorly as to escape counting. They considered the errors compensating and, therefore, made no deduction for split nucleoli. Davenport and Boethe (1934) deducted 3% from the total count to allow for split nucleoli. Duncan and Keyser (1936) made no deduction.

In the course of a study involving cell counts of the nodose ganglion the author undertook to determine the value of the error due to split nucleoli by the following method: From a given nodose ganglion were cut serial transverse sections in which the sections varied in thickness. Thus in the case of one ganglion, four sections were cut at 12 μ , then six at 9 μ , and finally eight at 6 μ . The process was repeated over and over again until the entire ganglion was sectioned. The total thickness of the group of sections of varying thicknesses was only 150 μ , an interval sufficiently close to insure that the different sections were equally and adequately representative. Other ganglia were cut in a similar manner using different section thicknesses. The method of approach is based on the principle that the differences in the calculated number of nucleoli based on the sections of different thickness are due to split nucleoli. Thus, for a given longitudinal unit of the ganglion the number of nucleoli split in 6 μ sections would equal twice the number split in 12 μ sections for the reason that there are twice as many sections.

All ganglia were fixed in a mixture of saturated corrosive sublimate and concentrated formalin, 9:1. They were embedded in paraffin (56°-58°), excepting one which was double embedded in celloidin-paraffin. The ganglia were cut on a new Spencer Rotary Microtome, Model 820, with a knife in excellent condition. The sections were stained either with thionin or Azure II-eosin. The counts of the nucleoli were made with a binocular microscope under high dry. A rectangle was marked with a diamond point pencil on a cover slip cut circularly to fit in the ocular. This rectangle, fixed in the ocular and oriented so that its long sides paralleled the lateral excursions of the mechanical stage, served to divide the sections into strips for counting, a method identical in principle with the method described by Davenport and Barnes (1935). Extreme care was used to avoid counting twice or failing to count nucleoli close to the border line. On thick sections the high power adjustment was continually used to bring all nucleoli into focus.

In the case of each ganglion, counts were made from sections of each thickness. Never less than three consecutive sections from each group were counted and sometimes four in the thinner sections.

In addition 300 cells were examined in serial sections of different thickness in the paraffin embedded ganglia to determine the number of cells showing nucleoli in more than one section. And 30 nucleoli selected at random were measured to determine the average diameter.

RESULTS

The results of the nucleolar counts are given in Table 1. The first four columns are self-explanatory. Column 5 will be explained later. Column 3 shows that the number of nucleoli actually counted is in each case equal to from $\frac{1}{4}$ to $\frac{1}{5}$ of the total number—a sufficiently large sample upon which to base the calculation of the total number. Column 4 indicates the calculated total of nucleoli based on sections

TABLE 1. COUNTS OF NUCLEOLI IN NODOSE GANGLIA MADE FROM SECTIONS OF DIFFERENT THICKNESSES. SEE TEXT FOR EXPLANATION OF FIGURES IN COLUMN 5

1 Ganglion	2 Thickness of sections (micra)	3 Number of nucleoli counted	4 Total number in ganglion (calculated)	5 Theoretical counts
1 (paraffin)	13	4,642	17,892	18,607
	8	3,330	17,953	
2 (paraffin)	12	6,841	28,504	29,216 30,694
	9	5,225	29,194	
	6	4,662	29,141	
3 (paraffin)	12	5,553	24,063	24,664
	9	5,462	23,668	
4 (celloidin-paraffin)	10	5,442	27,210	27,822 28,842
	8	4,197	26,231	
	6	4,458	27,862	

differing in thickness. It is at once obvious that considering their diameter, the number of nucleoli split is far less than the theoretical value. Measurements of 30 nucleoli established 3.5μ as the average diameter. Theoretically, in tissue sectioned at 10μ , $\frac{1}{3}$ of all nucleoli would be cut by the knife tho not thru their centers. It seems reasonable to assume that any nucleolus split anywhere in a center zone of a width of 1μ would be divided into two parts each of which would be large enough and stain well enough to be seen. In 10μ sections, since 1μ is $\frac{1}{10}$ of 10μ , 10% of all nucleoli would be cut thru a center zone of 1μ . Ten percent of the nucleoli would then be counted twice resulting in an error of 10%. In 6μ sections the error would be

16 $\frac{2}{3}$ %, i. e. thinner sections would increase the error. In column 5 are registered the different counts one could expect on the above assumption. The figures were obtained by applying the ratios of the theoretical values for sections of the different thicknesses to the figure actually obtained from the section of greatest thickness. The counts actually obtained from the various sections are in every case lower than the theoretical value.

Analysis of the calculated totals (column 4) indicates that the thickness of the sections did not affect the count. In the case of ganglia No. 2 and No. 4 the counts from the thinner sections are 2.0 and 4.0% higher, respectively. But in the case of ganglia No. 3 and No. 4 there are two instances in which the thinner sections give the lower count. In the case of ganglia No. 2 and No. 4 there should exist a progressive increase in the counts from those based on the thickest to those based on the thinnest sections, if split nucleoli do affect the count. No such relationship exists and the cases in which the thinner sections give the higher count are matched by cases in which thinner sections give the lower count. Altogether the data available indicate that thinner sections do not result in higher counts.

The examination of cells in serial sections of different thicknesses supports the above conclusion. Of 100 cells examined in paraffin sections 6 μ thick, there were three which showed nucleoli in two sections; of 100 cells in 9 μ sections, there were two; of 100 cells in 12 μ sections there were two. The results here show again that nucleoli do not split according to the theoretical expectations. It seems likely to the writer that a nucleolus is harder than the surrounding tissues or the paraffin and is pushed aside by the microtome knife into one or the other of the two sections the knife has separated. Evidence for this is sometimes seen in nucleoli that are misplaced. The results given just above, however, do suggest that there is an error of from 2 to 3% due to split nucleoli. But some of the pieces of the divided nucleoli observed were obviously pieces and would escape counting. Furthermore, the data from the examination of cells in serial sections is not as significant as the comparisons between counts based on sections of different thicknesses of the same ganglion. For, whatever the reason, if counts from thin sections do not differ appreciably from counts from much thicker sections, then split nucleoli are not affecting the counts in the thin sections and, therefore, not in the thick sections.

Differences in the counts, however, do exist tho they are of such character that, as pointed out, they cannot be interpreted to be due to split nucleoli. It is probable that the differences are due to the

lack of uniformity in the distribution of the nucleoli. Counts of adjacent sections differ from 0.0 to 10%. Repeated counts on the same section which constitute an evaluation of the personal factor differ from 0.0 to 2.0%. This error is, of course, compensating and is reduced by the large number of sections counted. The average of all the differences in the calculated total counts is somewhere near 2%.

CONCLUSION

No correction should be made for split nucleoli if the sections in which the nucleoli are counted are around 10 μ thick and none but distinct and definite nucleoli are counted.

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THE DIOXAN-PARAFFIN TECHNIC FOR SECTIONING FROG EGGS

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Probably every teacher of vertebrate embryology has experienced difficulty in obtaining suitable sections of the early developmental stages of the frog egg. These eggs are difficult to section due to the large amount of yolk present which becomes very hard and brittle after alcohol-xylene dehydration and are very apt to crack in sectioning. In this laboratory, remarkable success in sectioning frog ova and embryos has been attained by the use of dioxan (Eastman Kodak No. 2144) as a dehydrating and clearing agent. Not only do individual eggs (blastulae and gastrulae) section easily after this treatment, but large pieces of the frog ovary containing numerous ripe ova may be sectioned with little or no cracking of the sections.

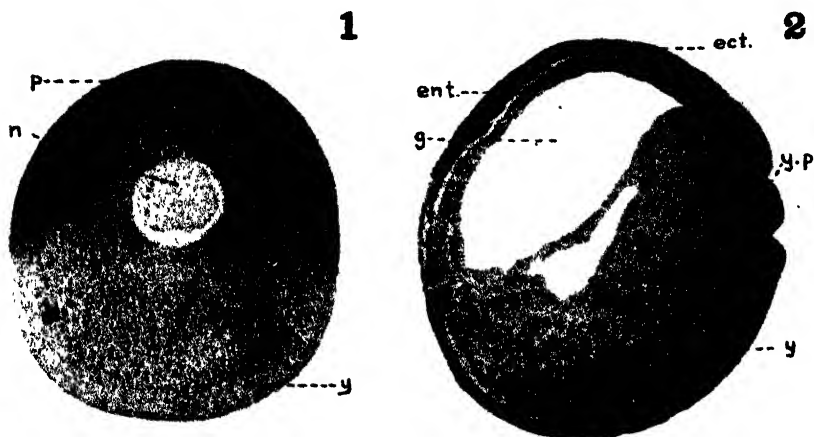


Fig. 1. Section of ovarian egg of *Rana sylvatica*. *n*, nucleus; *p*, animal or protoplasmic pole; *y*, vegetal or yolk pole. $\times 50$.

Fig. 2. Median sagittal section of a late gastrula of *Rana sylvatica*. *ect.*, ectoderm; *ent.*, entoderm; *g*, gastrocoele; *y. p.*, yolk plug. $\times 50$.

Figures 1 and 2 show sections made in this laboratory using the dioxan technic. Fig. 1 shows a section of the ovarian egg of *Rana sylvatica* and when studied microscopically shows very beautifully the structure of this telolethical egg. Fig. 2 shows a median sagittal section of a gastrula of *Rana sylvatica* and here again is obtained a very clear picture of the regional differentiations of the early embryo without any appreciable cracking of the yolk mass.

The technic employed in making the sections just described is essentially like that described by McClung¹ and will be briefly outlined as follows:

1. Fix piece of frog ovary (10–12 mm. square) or a single embryo in a mixture of one part of dioxan with two parts of Bouin's fluid.
2. Wash in dioxan.
3. Dehydrate and clear in fresh dioxan.
4. Infiltrate as follows:
 - (a) A warm mixture of 25 cc. of dioxan, 5 cc. of xylene and 20 cc. of soft paraffin (m. p. 50–52°).
 - (b) Soft paraffin (50–52°).
 - (c) Hard paraffin (53–55°).
5. Embed in hard paraffin (53–55°).
6. Section at 10 to 12 μ , spread and allow to dry.
7. Decerate in xylene.
8. Pass sections thru absolute, 95%, 80%, and 70% alcohol.
9. Stain in Delafield's hematoxylin in the usual manner.
10. Wash stained sections in tap water, dehydrate in the usual manner (destaining sections in 70% acid alcohol, if necessary).
11. Clear in xylene and mount in Canada balsam.

¹McClung, C. E. 1936. A dioxan technic. *Stain Techn.*, **11**, 121.

THE USE OF BUFFERED SOLUTIONS IN STAINING: THEORY AND PRACTICE¹

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ABSTRACT.—The importance of pH in staining tissue is emphasized. The effect of pH upon the selectivity and intensity of staining with iron hematoxylin, malachite green, and eosin Y is considered. Many difficulties may be avoided by staining in the higher alcohols and directions are given for the preparation of buffer solutions from pH 1.2–8 in alcohol. The concentration of stains, time of staining, and order of staining are discussed for progressive and regressive staining. At pH 8 in 95% alcohol very few tissues stain with malachite green at a concentration of 1/1000 saturated. At pH 6 most cytoplasmic elements stain with malachite green at a concentration of 1/1000 saturated or with eosin Y at 1/250 saturated. As the pH is lowered more tissue elements stain until the nucleus is completely stained. This behavior is in accord with the theory of chemical combination of dyes with proteins, which states that proteins combine with basic dyes on the basic side of their isoelectric points and with acid dyes on the acid side of their isoelectric points. With hematoxylin stain the pH range is much shorter. A satisfactory hematoxylin stain is composed of 0.1% hematoxylin, 0.1% FeCl₃, and HCl to bring the pH to 1.2–1.6 in 80% alcohol. With this stain, which may be used immediately, the nuclei of most tissues begin to stain at pH 1.2 and much of the cytoplasm will be stained if the pH is raised to 1.4. The shortness of this effective pH range is thought to be due to the dissociation of the hematoxylin-iron-protein complex. The use of different dyes successively at different pH values, such as hematoxylin at 1.3, malachite green at 8, and eosin at 6, permits better differentiation of the tissue elements, and intelligent variations in the staining technic.

Among the more important factors involved in the process of staining biological material one might list: the character of the tissue, the chemical nature of the stain, and the concentration of this stain. Time, of course, is a factor, but it is effective primarily as a function of concentration and penetration. The character of a given tissue is the expression of a definite combination of the several constituents

¹From the Laboratories of Insect Physiology and Toxicology, Division of Entomology and Parasitology, University of California.

present. Its chemical behavior may be greatly modified by loss or chemical alteration of one or more of these respective constituents, a process which may occur during the preliminary preparation of the tissue for staining. The character of the stain, in the ideal case, is relatively constant but may be altered by the use of a mordant, or, in the cases in which the stains are acidic or basic substances, by changing the pH of the staining solution. The concentration of the stain must be sufficient to cause the formation of enough of the tissue-dye complex to give a recognizable color. The concentration of the active part of the dye may be altered by changing the pH since the degree of ionization of the dye acid or base will be altered. It is evident that pH plays a part in affecting each of the factors involved in staining. The control of pH is especially important when slight differences in homologous tissue elements are being studied and when a pathological tissue is being compared with a normal.

It is the purpose of this paper to summarize the effect of pH on the character of the stained preparations and to present a simple method giving precise, reproducible staining at various indicated pH values. This method has been in use for more than a year in our laboratory and in the zoological laboratories of this university and of Stanford University with considerable success.

Experimental. It is a common practice in staining to vary the pH in a more or less controlled fashion by the addition of acids, carbonates, ammonia, and similar substances. The reactions in more or less well buffered solutions have been studied by many investigators, especially Stearn and Stearn (1928), Yasazumi (1935), and Kelly and Miller (1935). In following the procedures given in the literature for staining with controlled pH, certain difficulties have been encountered. The solutions used are frequently only adjusted to a definite pH and no provision is made for maintaining it during subsequent additions of material. While there are adequate buffer systems for aqueous solutions, we have found, as have many others, that the subsequent washing, dehydrating, and clearing operations extract so much of the stain that precise differentiation is impossible. The obvious way to avoid the washing out of dye is to stain in the higher alcohols. There are some procedures using alcohol in which the pH is fixed but usually unknown, and so far as can be ascertained there is no method in use in which the pH in alcoholic stains can be held at any predetermined value. The substances selected for buffers were acetic acid and its derivatives for the high pH range and hydrochloric acid for the low pH values.

Preparation of the Buffer Solution. Since the solvent is alcohol, the

dissociation constants of the acids as determined for water will be too large (Michaelis and Mizutani, 1925). The dissociation constants in alcohol were ascertained by determining the pH of mixtures of approximately 1.0 *N* acid and base in water diluted with absolute alcohol to a concentration of alcohol of 95%. The resultant anion concentration was about .08 *N*. The pH of each solution was determined by means of both the hydrogen and glass electrodes. The pKa values obtained are: acetic acid, 6.9; monochloroacetic acid, 5.0; and trichloroacetic acid, 2.2; all in 95% alcohol. For the staining experiments, stock solutions of the acetic acid series and sodium hydroxide were made 0.1 *N* in 95% alcohol and mixed in the desired proportions. The proportions can be readily calculated by the Henderson equation:

$$\text{pH} = \text{pKa} + \log \frac{(\text{base added})}{(\text{initial acid} - \text{base})}$$

or from the curves in Fig. 1. For very acid solutions hydrochloric

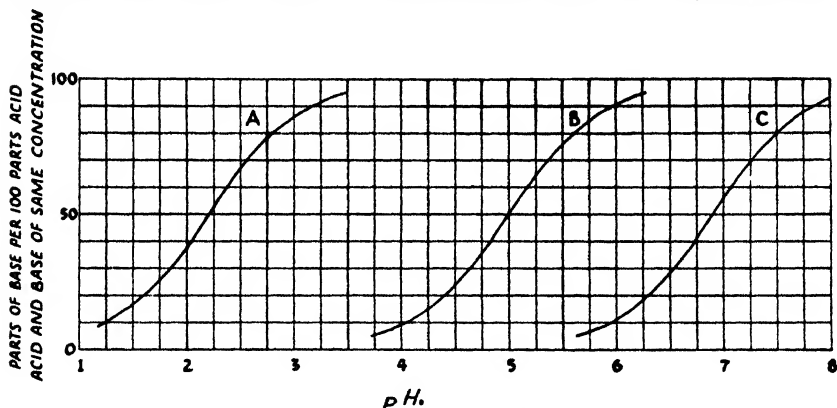


Fig. 1. Buffer curves. A, Trichloroacetic acid. B, Monochloroacetic acid. C, Acetic acid. Acid and base in 95% alcohol.

acid in 80% alcohol was used. Its pKa value is about 1.5. The concentration of hydrochloric acid for the pH desired can be calculated from Fig. 2. There is some decomposition of the stock solutions of the chloroacetic acids so that it is best not to depend upon them for more than a few weeks. Altho the pKa values of the acids in alcohol given above are provisional, any future change in their values will not affect the usefulness or validity of the results obtained using the values given here.

Preparation of the Stock Solutions of Stains. The stains used were malachite green, C. P. (Eastman Kodak Co.), eosin Y (Coleman & Bell; and National Aniline Co.) and hematoxylin (Eastman Kodak

Co.; and MacAndrews & Forbes). The malachite green and eosin were made up in two or three times saturated solutions in 95% alcohol, centrifuged, and the saturated solutions of stain decanted. This gives a stock solution saturated with respect to the active ingredient. Dilutions of these stock solutions will be referred to as S/100, S/1000, etc. (which is to be interpreted as one part saturated solution of dye per 100 or 1000, etc.).

Preparation of Materials. The materials used were grasshoppers, onion root tips, and paramoecia. The paramoecia were fixed in Schaudin's solution, and the root tips in Karpechenko's solution (see Rawlins, 1933). Male grasshoppers were fixed in one of the following solutions: 2% potassium bichromate, 10% formalin, 100%

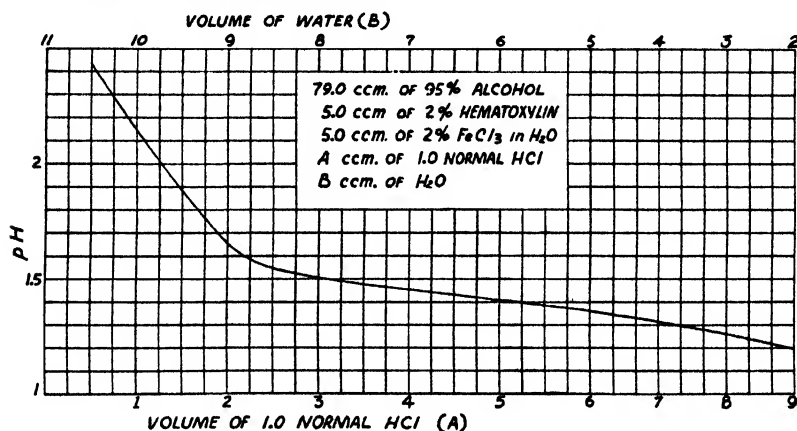


Fig. 2. pH values for hematoxylin staining mixture versus amount of 1.0 normal HCl.

alcohol, Bouin's with urea, (Lee, 1928), and Petrunkevitch's cupric phenol (Petrunkevitch, 1933). The grasshoppers and root tips were sectioned longitudinally in paraffin. Approximately corresponding sections of each of the grasshoppers were mounted on the same slide, making five sections per slide. The paramoecia were handled in bulk. Following the establishment of useful pH values and dye concentrations on the above tissues, many other materials have been stained, such as nematodes, Ophryoscolecidae, *Lumbricus terrestris*, Trychonympha, normal and cancerous mouse mammary tissue, lymph node (mouse) and many species of insects.

Hematoxylin. Solutions of hydrochloric acid of pH 1.65, 1.45, 1.35, and 1.25 were made up in 80% alcohol (see Fig. 2); ferric chloride and hematoxylin to the amount of 0.1% each were added and the tissues stained over night. They were then rinsed for 1-2 minutes in 90%



Plate I. Effect of pH on density of iron hematoxylin stain. Top to bottom, pH 1.23, 1.39, 1.46. Staining mixture as given in Fig. 2.

alcohol, blued in slightly less acid (pH 4-8) 95% alcohol, cleared in carbolxylene and mounted. The ferric chloride solution must be freshly prepared since the colloidal form is not usable. The stain may be used as soon as mixed and will keep for several days.

Counterstains. For the counterstains, acetate buffers at pH 4, 6, and 8 were made up in 95% alcohol (see Fig. 1). Suitable concentrations of counterstains for 12 hour staining were found to be S/1000 for malachite green, and S/250 for eosin, in 95% alcohol. After staining the slides were rinsed 1-2 minutes in 100% alcohol, cleared in carbolxylene and mounted.

		HEMATOXYLIN			MALACHITE GREEN			EOSIN Y		
		pH 1.2	pH 1.4	pH 1.5	pH 4	pH 6	pH 8	pH 4	pH 6	pH 8
GANGLIA	N	●	●	●	●	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
FAT	N	●	●	●	●	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
HYPODERMIS	N	●	●	●	●	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
MUSCLE	N	○	○	○	●	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
MIDGUT	N	●	●	●	○	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
OENOCYTES	N	○	○	○	○	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
TUBULES	N	○	○	○	○	○	○	○	○	○
	C	○	○	○	●	○	○	○	○	○
INTEGUMENT	ECTO.	○	○	○	○	○	○	○	○	○
	ENDO.	○	○	○	○	○	○	○	○	○

Table 1. Relative intensity of staining of grasshopper tissues fixed in Petrunkevitch's cupric phenol. N, nucleus, C, cytoplasm.

Analysis of Results. Precautions were taken in photographing the slides to secure an accurate record of the differences, Plate I. Results of visual observations of eight different tissues of grasshoppers fixed in Petrunkevitch's cupric phenol are given in Table 1. The other fixing agents gave essentially the same results.

Discussion and Results. There are three principal types of chemical compounds comprising protoplasm which may be stained—fats, carbohydrates, and proteins. The fats and carbohydrates react only with special types of stains or dyes. Among the proteins are included the glycoproteins or protein sugars and the lipoproteins or

fatty proteins which are substances that may react with the usual nuclear and cytoplasmic stains. For this reason only the proteins will be considered in this paper and that term will be used to include the compound as well as the simple proteins.

Altho due cognizance was taken of the two general theories of staining reactions, the one assuming chemical combination, (Stearn and Stearn, 1928; Rawlins and Schmidt, 1930) and the other adsorption, we have emphasized only the former. Stoichiometrical combination can account for the facts observed, and it has proved a useful theory upon which much serviceable practice has been built. Tho many of the phenomena observed can be accounted for by either theory, the presentation of a coherent discussion seems to indicate a consideration of only the theory of chemical combination.

The active groups in a protein molecule are the amino, carboxyl, and peptide linkage. The peptide linkage is less reactive than the others. A fourth group which is concerned in the reaction with iron and possibly other metals, is the OH group of the dicarboxylic acids and the hydroxy acids.

The dyes used in staining are generally acidic or basic in character, the acidic groups being the carboxyl, the phenyl-hydroxy, the sulfonic acid, and certain less important ones such as chlorides, nitrates, etc., which accentuate the acid character. The basic group is the amino group modified by substituents.

The reaction between a dye and a protein has been assumed by many workers to be salt formation. If the reaction is between an acid dye and a protein, then the protein must be in the basic state and the reaction is between the amino group of the protein and the carboxyl group of the dye. If the reaction is with a basic dye, then the reaction is between the amino group of the dye and the carboxyl group of the protein.

If the pH of the medium is basic to the isoelectric point of the protein, the latter will combine with a dye cation or basic dye, whereas if the pH is acid to the isoelectric point of the protein, it will combine with a dye anion or an acid dye. In alcoholic mixtures the isoelectric points of most proteins lie between 2 and 8, those of the proteins in the nucleus between 2 and 4, and those of the proteins in the cytoplasm between 4 and 8, (Jukes and Schmidt, 1934). These are general values, and exceptions may readily be found. If the pH of the solution is fixed at 4, then all proteins whose isoelectric points are less than 4 will stain with a basic dye. At this pH, acid dyes would be expected to stain the cytoplasm, but not the nucleus, while basic dyes would stain the nucleus, but not the cytoplasm. Actually, there is

considerable overlapping since the cytoplasm may contain some more acid proteins and the nucleus some more basic proteins. In general there will be many proteins of different isoelectric points in any tissue and its acidic or basic character is determined by the average value of the isoelectric points.

In staining cytoplasm, use may be made of the isoelectric points of the proteins to carry out selective staining. Thus at a high pH, such as 8, practically no tissue will stain. As the solution is made more acid, more of the tissue elements will stain until all of the cytoplasm and some of the nucleus is stained. It is not possible to stain the nucleus with an acid dye without staining the cytoplasm. The entire tissue may be stained and then treated with the buffered dye which will remove stain from all proteins whose isoelectric points are less than the pH of the buffer; thus the nucleus may be destained.

To stain the nucleus alone, use a basic dye which will combine with more and more proteins the more basic the solution, until the nucleus is completely stained. The usual dye bases are the amines. The amino group, however, has a strong tendency to add hydrogen ion, and acts as an anion, usually combining with proteins acting as cations. The common stain for nuclear material is hematoxylin mordanted with iron or aluminum. There are two ways in which a mordant may act. There may be an insoluble compound formed between the protein and the mordant. In this case the mordant is allowed to penetrate the tissue until the desired concentration has been built up, and then the dye is applied. As the dye penetrates the tissue, an insoluble dye-mordant compound is formed which is called a "lake". In the other case the dye and mordant form a new compound which is used to stain the tissue.

Hematein, the active principle of hematoxylin, is a hydroxy compound which unites rather readily with iron. Since iron (Smythe and Schmidt, 1930) is known to react with protein, it seems reasonable to assume that it forms a complex in which iron binds the dye to the protein. If the iron is bound by the ionic form of the protein, the amount of iron combined will increase with the pH to some extent. From Smythe's work it appears that this increase is not great. There is, however, the effect of pH on hematein which may provide a working hypothesis. If the strength of the bond between the iron protein complex and hematein increases with pH, then at low pH the stain will be very selective and on raising the pH the amount of hematein bound to the tissue will increase until the hematein is precipitated by the iron. The facts are that at any given concentration of hematein the density of stain is remarkably sensitive to changes in pH.

There are many ways of using hematoxylin stains. One of the simplest is to make a solution of hematoxylin in 80% alcohol containing ferric chloride and sufficient hydrochloric acid to bring the mixture to the desired pH. This solution may be used immediately. Apparently the ferric chloride, besides acting as a mordant, serves to oxidize the hematoxylin and thus to ripen it.

In the usual procedure for staining with hematoxylin in aqueous solutions the principle function of the alum is to fix the pH at about 3.2. If a buffered aqueous solution is used, then either iron or aluminum nitrate or chloride may be used. The color of the stain as well as the specificity depends largely on the pH. A low pH makes for very selective stain, but gives a reddish color. This may be changed to blue by bathing the slide in a slightly more basic solution, approximately pH 4 to 8. Hematein without a mordant is a generalized stain. The effect of pH on the staining of several tissues is shown in Plate I and given in detail for the Petrunkevitch-fixed grasshopper in Table 1. The effect on the root tips and paramoecia is not nearly so marked since their tissue elements are more homogeneous and require a greater spread of pH to show equal differences in staining. Thus at pH 1.7 the root tips are very heavily stained.

Since the acid dyes are used in relatively basic solutions, and the basic dyes in relatively acid solutions, the dye will usually be completely dissociated. The acid dyes, however, may be used in a solution whose pH is as low as 4, in which case a weak acid would not be completely dissociated and less staining would result. This is exemplified by eosin Y, which stains less at pH 4 than at pH 6 (Table 1).

Since the amount of dissociation of protein and dye into ions is fixed by the pH of the solution, the amount of combination at a given pH is controlled by the concentration of the dissociated dye and the time of staining. The rate of staining, or more specifically the rate of reaction, is a function of the permeability of the tissue and varies greatly with the dye used. To show the chemical differences in the tissue elements, the best results are given by a long staining time, approaching equilibrium, which may vary from one-half to twelve hours. The amount of combination, or depth of staining, increases directly with the concentration of the dye. The pH is also very important because it controls the effective concentration. The amount of the protein in the ionic form depends upon the pH, and since a small change in pH makes a large change in protein ion concentration near the isoelectric point, changing the pH by one unit may change the amount of proteins in a stainable condition many fold. For malachite green a shift from pH 8 to pH 4 will cause an increase in over-all

density roughly comparable to a ten-fold increase in dye concentration at pH 8. An equal change is produced in the amount of dye protein compound for a change in either the concentration of ionized dye or protein ion. If the dissociation range of the dye happens to coincide with the isoelectric point of the protein, then the pH will be a controlling factor for both the concentration of the ions of the dye and of the proteins.

For emphasis of particular tissue elements, such as chromosomes, overstaining followed by destaining may be useful. This may be accomplished in several ways. When staining chromosomes with iron hematoxylin, (a) the pH giving best differentiation may be chosen and the tissue overstained by increasing the concentration, then destained in a normal concentration at the same, or even better, at a lower pH; (b) the tissue may be overstained in a relatively alkaline solution, using either the usual or higher concentrations of stain, and then destained in a pH giving best differentiation or in one more acid. Rapid staining with iron hematoxylin can be carried out either by increasing the concentration of the stain, by increasing the pH, or by both. Better results are obtained by increasing the concentration.

When the nucleus is stained first in an acid medium with a basic dye and the counterstain is an acid dye used in a relatively basic medium, there is no danger of washing out the nuclear stain. Iron hematoxylin does not wash out in 48 hours. The counterstains may or may not wash out, depending on the amount of dissociation of the dye-protein combination. Thus eosin washes out easily and malachite green very slightly.

When two or more counterstains are desired, the process is very simple. As an example, one first determines the concentration of eosin which will give a good picture at pH 6 and the concentration of malachite green which will give a good picture at pH 8. The tissue is then stained in iron hematoxylin, rinsed in 90% alcohol, stained in malachite green at pH 8, rinsed in 95% alcohol, stained in eosin at pH 6, rinsed in 95% alcohol and absolute alcohol, and cleared in the usual manner. Eosin is used last since it washes out the most readily. This method will give the minimum of overlap of the two stains since only part of the tissue is stained at pH 8 and part at pH 6, while a third counterstain may be used at pH 4. Usually the tissue elements contain a mixture of proteins so that the colors are intermingled and cannot be separated. This effect is less noticeable under high power where frequently the colored granules may be resolved. For example, striated muscle which is usually stained in bands may be stained in alternate strips of color.

Overstaining and destaining with counterstains is very feasible. The same methods as outlined for hematoxylin apply, except that, since the effect of pH is reversed, the material is overstained in acid and destained in alkaline solutions.

Those who wish to extend this method to other stains or solvents must remember that only substances which are soluble and ionized in the solvent can be used. The range of ionization must be in the useful pH range.

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PERMANENT FEULGEN SMEARS FROM PREVIOUSLY FIXED MATERIAL

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In 1936 Heitz¹ published a paper on the Feulgen technic of staining chromosomes, which implies an improvement worthy of more general attention. After fixation in chromic-osmic acid for 15 minutes or more, hydrolizing in normal hydrochloric acid 10–30 minutes, according to length of fixation, and treating with Feulgen stain solution for 30 minutes or more, the material is transferred to a drop of 45% acetic acid on a slide and mechanically disintegrated. The acetic acid prevents the cytoplasm from becoming stained red by fuchsin, a disadvantage of the Feulgen method which has been difficult to avoid altogether. The tissues are then slightly flattened out by gently pressing on the cover glass.

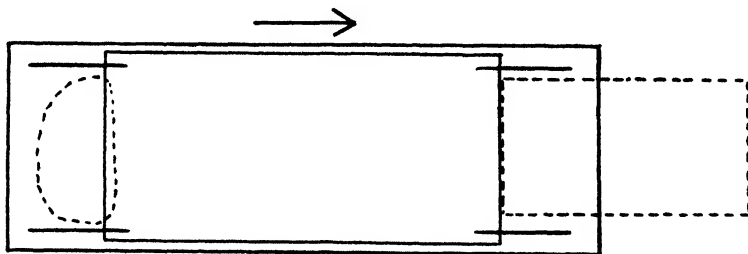


FIG. 1.

I have successfully used the technic for grasshopper testes fixed during the summer and stored in 80% alcohol. The material was run down to water (80%, 70%, 50%, 30%, 10% alcohol and water, 5–10 minutes in each), and placed in a normal hydrochloric acid solution, kept at a temperature of 57°–60° C. by placing in a paraffin oven for 25 minutes. It was then transferred directly to the Feulgen stain solution for 1–6 hours. It was next disintegrated in a drop of 45% acetic acid by tearing with needles and tapping with the edge of a scalpel. All unnecessary debris should be removed before applying the cover glass. Slight pressure was then applied by blotting carefully to spread out the cells.

¹Heitz, E. 1936. Die Nukleal-Quetschmethode. *Ber. deut. botan. Ges.*, 53, 870–8.

The best results were obtained with material fixed in Navashin. Also material fixed in alcohol and acetic acid, 3:1, was used, but from water it was transferred to 1% chromic acid for 1-2 days and soaked in warm water on top of the paraffin oven (temperature about 30° C.) for one hour before hydrolizing.

There was, however, no satisfactory method to make the preparations permanent. Technics which involved removal of the cover glass lead to the loss of most of the material. It was then found, however, that the changing of fluids underneath the cover glass is easily performed in the following way: A long thin cover slip is used. Both ends are raised with the aid of very thin needles as shown in Fig. 1. I have used so-called "Minutienstifte" which entomologists use for pinning very minute insects. Most of the length of the cover slip remains close to the slide and holds the material between the glass surfaces. The raised ends easily allow the fluids pipeted at one end to be sucked out with blotting paper at the other end. The balsam should, of course, be very thin. (I have found the McClintock series for making aceto-carmin preparations permanent to be quite useful here also, which is not surprising as in both cases we are dealing with acetic acid at the start.) Then come: absolute alcohol and glacial acetic acid, 1:1; absolute alcohol and glacial acetic acid, 3:1; absolute alcohol and glacial acetic acid, 9:1; absolute alcohol; absolute alcohol and xylene, 1:1; and eventually balsam. In cases where the cells are liable to shrink, absolute alcohol, glacial acetic acid, and water, 1:1:1, may be inserted after 45% acetic acid with advantage.

In successful preparations whole groups of cells at the same stage are spread out in a single layer and have retained their natural form without any damage. Naturally a few cells will be damaged and free chromosomes are frequently seen in the preparations, but this only gives an opportunity to study the latter in more detail.

THE USE OF N-BUTYL ALCOHOL IN THE PARAFFIN METHOD

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ABSTRACT.—Several modifications in the use of *n*-butyl alcohol are suggested. These modifications include a revised series of dehydration solutions for exacting work, an abbreviated schedule of limited usefulness, and a simple method for more rapid paraffin infiltration. The use of a triangular coordinate graph may be valuable in designing dehydration procedures for special purposes. Changes in the primary fixation image are significantly less severe by dehydration with butyl alcohol than with many other reagents. Such deleterious effects may be further minimized by reducing the time and temperature factors to the practical limit and by substituting acetone for ethyl alcohol in a dehydration series such as that of Zirkle.

N-butyl alcohol has found a prominent place in the paraffin method largely thru the recommendations made several years ago by Zirkle.¹ Many other dehydration-infiltration reagents have been suggested in the literature and among these are acetone, chloroform, trichloroethylene, petroleum ether, diethyl ether, methyl benzoate, terpeneol, benzene, toluene, methyl salicylate, cedarwood oil, ethyl, methyl, and butyl acetate, the propyl alcohols, the secondary, iso, and tertiary butyl alcohols, several pentanols, and dioxan. Several efforts have been made to ascertain the relative merits of a restricted number of these chemicals for paraffin work. For many cytological purposes and for general use also, *n*-butyl alcohol is perhaps one of the most satisfactory dehydration-infiltration chemicals now in use. As with other dehydration chemicals, the excellence of the results may vary with the method of use, and the purpose of the present paper is to suggest several modifications in schedule which have given improved results in both cytological and anatomical work.

The basis for the present recommendations may be noted in Fig. 1. On this graph are shown all possible proportions between water, ethyl alcohol, and *n*-butyl alcohol and any one point on the graph represents a solution of a given composition. Since water and butyl alcohol are only partially miscible, two-phase or conjugate solutions will exist when water, butyl alcohol, and ethyl alcohol are mixed in cer-

¹Zirkle, Conway. 1930. The use of *n*-butyl alcohol in dehydrating woody tissues for paraffin embedding. *Science*, 71, 103-4.

tain proportions. In Fig. 1, all points which lie on or below the lowest or binodal curve represent those proportions which give rise to such two-phase systems and these solutions should be avoided for a dehydration schedule such as that of Zirkle. All points above this curve represent one-phase or homogeneous systems and these solutions are potentially useful for dehydration purposes. A set of dehydration solutions is obtained when points above the binodal curve

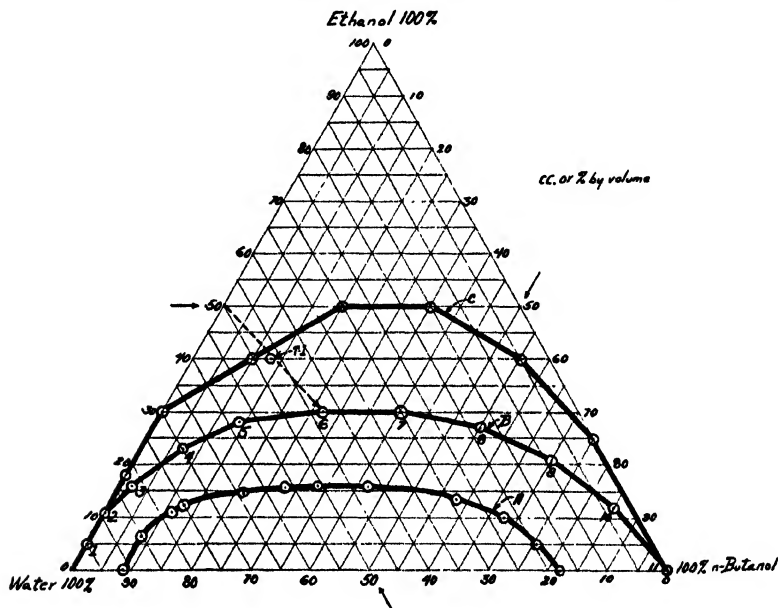


Fig. 1. A graph illustrating all possible proportions between water, ethyl alcohol, and *n*-butyl alcohol. The binodal curve, *A*, represents the lower limits at which homogeneous systems occur at 25° C. The upper curve, *C*, represents the dehydration series recommended by Zirkle. The middle curve, *B*, is recommended for general cytological purposes. Curves as low as 20% ethyl alcohol may be used with savings in time if points on the curve are selected with care.

are selected in sequence from the lower left-hand corner (pure water) to the lower right-hand corner (pure butyl alcohol). The shortest path between the basal extremities of the graph would represent a theoretically ideal course for dehydration with *n*-butyl alcohol. This is true because the amount of liquid interchange to which material is subjected reaches its minimum in a flat curve. An example between the possible extremes will illustrate this point. If material is transferred into 100% ethyl alcohol in 10 steps and then transferred into pure butyl alcohol in 10 steps, the average interval will be 10% and the total liquid interchange may be designated as 200%. In addi-

tion, the 100% dehydration will have been attained in the first 10 steps of this procedure. If, however, the material could be transferred in the same number of steps from water into pure butyl alcohol without the use of ethyl alcohol, the average interval and the average dehydration would be 5% and the total liquid interchange would be 100%. On this basis and independently of other considerations, it will be seen that a 7-step dehydration series based on a curve with a 20% maximum ethyl alcohol concentration should afford results comparable to those from a 10-step series based on a curve with a 50% maximum ethyl alcohol concentration. For most purposes, therefore, it seems desirable to make use of a flat curve in which the ethanol concentration does not exceed 25 or 30%. It is obvious that the curve in any instance merely indicates a course by which dehydration may be accomplished and that the points selected on this curve will designate the composition of the dehydration solutions which are to be used.

TABLE 1. EXPERIMENTALLY DETERMINED POINTS FOR THE CONSTRUCTION OF THE BINODAL CURVE SHOWN IN FIGURE 1. TEMPERATURE 25° TO 27° C.

	1	2	3	4	5	6	7	8	9
Water	42.2	28.9	22.4	50.3	56.1	18.8	13.7	64.1	75.2
N-Butanol	42.2	57.8	67.3	33.6	28.1	75.5	82.0	21.4	12.5
Ethanol (Abs.)	15.6	13.3	10.3	16.1	15.8	5.7	4.3	14.5	12.3

A satisfactory series for general cytological work is shown on curve *B* in Fig. 1 (see also Table 2). This series has been used with satisfactory results on representative cytological materials among the major plant groups following certain kinds of fixation. Following those types of fixation which are particularly destructive of the cytoplasm, that is extreme "nuclear" types, it will be found desirable to add intermediate steps by mixing adjacent solutions, at least in the first half of the series, for here the materials are most easily damaged because of their softness.

The use of a graph such as that of Fig. 1 will simplify the dehydration of materials fixed or preserved in alcoholic solutions. If this solution is 50% alcohol, for example, a line drawn from that point on the graph to the nearest solution of the dehydration series will suggest the best possible route by which material can be dehydrated further, and one or more suitable points as transition solutions may be chosen on or near this line (Fig. 1, Table 1).

Most of the modifications of the Zirkle series which have been recommended in the literature concern abbreviated schedules for special types of work. The use of a graph may be found valuable in

designing short schedules for such work. An example of limited usefulness is offered. When butyl alcohol is thoroly saturated with water, the solution has a negligible dehydrating effect. Plant material in aqueous media may be placed into such saturated butyl alcohol (approx. 82%) without experiencing the slightest distortion. If the material is supported in the upper phase of a saturated mixture, the material will, in time, become saturated with 82% butyl alcohol. From this point the material may be further dehydrated in one or more steps without the introduction of ethyl alcohol. Another method is to place material which has become permeated with 82% butyl alcohol directly into the paraffin oven. In this instance, since the vapor pressure of water is significantly higher than that of butyl alcohol, the water will be removed from the solution when the liquid has evaporated to approximately $\frac{1}{2}$ to $\frac{1}{3}$ of its original volume and paraffin may be added at this point to effect infiltration in the usual manner. Good results can be obtained even with very fragile materials if one has time available for experimentation. This procedure has been used primarily with material fixed in saturated butyl alcohol to which 4% formalin and 4% formic acid has been added. This fixing agent, when thoroly saturated with water, has proved valuable as a fixing and preserving fluid for certain tissues which are especially susceptible to damage by ordinary fixatives. When desirable, material may be transferred from this fixing solution into 70 or 95% ethyl alcohol without injury to anatomical features.

In the experience of the writer, paraffin infiltration is most successfully carried out with a procedure which by description seems rather severe. Material in pure butyl alcohol is placed into an open preparation dish and is covered with as much liquid as the dish will conveniently hold. A paper bridge is arched over the material and enough paraffin is placed upon this bridge to just cover the material when the butyl alcohol has evaporated completely. The dish is then placed into a well-ventilated oven maintained at a temperature which will melt the paraffin. Material for cytoplasmic study is left in the oven for the minimum necessary time, especially if the fixation has been brief. Other materials may be left in the oven for longer periods of time according to the thoroness of the fixation and the results desired.

Apparently all dehydration reagents, in common, exercise certain deleterious effects upon the primary fixation image, particularly the tendency to dissolve portions of the fixed cytoplasm. Destructive effects of this kind are significantly less severe with *n*-butyl alcohol than with most other reagents and this feature constitutes perhaps

the outstanding merit of butyl alcohol for cytological work, especially in cytoplasmic studies or in comparative studies of fixation. The maximum benefits of this potential advantage are realized only when the time periods and temperature factors are kept at the practical minimum. Since ethyl alcohol is particularly destructive of fixed cytoplasm, further advantages are afforded by the use of a flat curve such as that of Fig. 1, since the concentration of ethyl alcohol is determined by the height of the curve. Another method of avoiding pronounced changes in the primary fixation image is to substitute acetone for ethyl alcohol in a dehydration series such as that of Zirkle or that of Table 2.

TABLE 2. THE COMPOSITION OF THE SOLUTIONS SHOWN ON CURVE B OF FIGURE 1

Solution No.	1	2	3	4	5	6	7	8	9	10	11
Water	95	89	82	70	57	43	30	18	9	3	—
Ethanol (95%)	5	11	16	23	28	30	30	27	21	12	—
N-Butanol	—	—	2	7	15	27	40	55	70	85	100
% Alcohol	5	11	18	30	43	57	70	82	91	97	100

It is obvious, of course, that the most thoroly preserved fixation image is not necessarily the most favorable for a certain type of study. There is perhaps no one "best" method of dehydration and it is necessary to select those reagents and that schedule which can be used to best advantage for a particular purpose.

CHROMATOGRAMS OF BIOLOGICAL STAINS ON ACID AND BASIC ADSORBENTS

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ABSTRACT.—A simple method of preparing chromatograms of mixtures of various biological stains on common acid and basic adsorbents is described. From the study of such chromatograms, three kinds of preferential adsorption can be recognized. (1) Due to the preferential adsorption of acid stains on basic adsorbent, a mixture of stains will separate itself into successive color bands on the chromatogram according to their individual acidity or basicity. The chromatogram, therefore, of a mixture of three stains—acid, neutral and basic—will appear on basic adsorbent according to the above order. The reverse order results if the acid adsorbent is used. The stains can be taken off from the adsorbents by shaking with proper solvents. (2) The components of some neutral stains or a mixture of some similarly charged stains can also be separated in a chromatogram. The order of appearance seems to be independent of the acid or basic nature of the adsorbents. This is similar to the chromatogram of leaf extract of plant pigments. (3) Finally, some mixtures of acid and basic stains do not follow their regular sequence of appearance on the chromatogram when adsorbed on magnesia. The reason for this anomaly is not clear, probably due to formation of adsorption complex.

This technic can be used to detect and separate mixtures of stains and to demonstrate the nature of adsorption and theory of staining. It can be used also as a preliminary test for the choice of solvent and adsorbent for chromatographic analysis. For the purpose of demonstration, an artificial cell can easily be made by impregnating talc (acid) and magnesia (basic) in collodion, in the form of nucleus, cytoplasm, etc., which is stained by following the general histological technic after the collodion is dried.

Biological stains are usually classified as acid, neutral and basic stains according to the nature of chromophore groups present (see Conn, 1936). The acid and basic nature of the chromophore groups can be shown by the capillary analysis. (See Zsigmondy, 1917 or Feigl, 1931.) Strips of filter paper are dipped in different dye so-

¹The author wishes to express his acknowledgment to Dr. E. S. Miller for his kind help and valuable suggestions.

lutions. The ascent of dyes to different heights is believed to be due to difference in the electric charge carried by the chromophore groups. Filter paper itself is considered as negatively charged. As a result, negatively charged chromophores (acid) rise with their dispersing media, while positively charged ones (basic) are held near the surface. If a mixture of the two oppositely charged dyes is dropped on a filter paper and then carefully spread out by adding a few drops of the solvent, the dyes will accordingly diffuse out at different rates until two zones will appear, one encircling the other, the outer zone being the negatively charged dye. If, instead of using filter paper, a tightly packed mass of some pulverized inert substance, such as talc or magnesia, is employed, the dyes will separate into definite colored zones next to each other, forming the so-called chromatogram in chromatographic analysis. The colored zones can be separated and the dyes redissolved by shaking with proper solvents. In other words, the technic of chromatographic analysis can also be adopted for the separation of biological stains.

Chromatographic analysis has long been recognized as a useful tool for the separation and purification of closely related organic compounds. (See Winterstein and Schön, 1924; Zechmeister and Cholno-ky, 1936.) Tswett (1906) first used it for the separation of chlorophyll and related pigments. The separation was attained by passing the leaf pigment extract thro a column of tightly packed adsorbent. In this way a chromatogram was obtained, and the different pigments were found separated in zones of the adsorbent. Altho recently this technic has been widely employed in the separation of related organic compounds, the exact mechanism of the preferential adsorption is still not known. Hence, the selection of solvent and adsorbent is quite arbitrary and empirical. The adsorbents most commonly used are water-insoluble oxides of magnesium, calcium, aluminium, and silicon (silicates of Al, Mg, etc., silica gel, infusorial and diatomaceous earth, kaolin, clay, fuller's earth, Lloyd's reagents, etc.). By applying the common adsorbents in the separation of known mixtures of stain solutions, several points of interest have been observed. These observations have enabled us to differentiate at least three different kinds of preferential adsorption. The nature of the stains as well as the adsorbent and the theory of staining can also be demonstrated by this technic.

It is known that many insoluble particles acquire electric charges when in contact with an aqueous solution. Hence, magnesia, MgO , in water is positively charged; while talc, $3\text{MgO} \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$, is negatively charged. By filtering a mixture of acid, neutral and basic

stains, such as methyl orange, Sudan III and anilin green in 95% ethyl alcohol, thru a column of well packed magnesia, a chromatogram of three colored zones appears: orange, red and bluish green. If the same solution is allowed to pass thru a talc column, the colored zones are reversed in order, the uppermost zone being green. Similarly for a variety of mixtures of basic and acid stains such as crystal violet-eosin, iodine-green-acid-fuchsin, basic-fuchsin-light-green, etc.

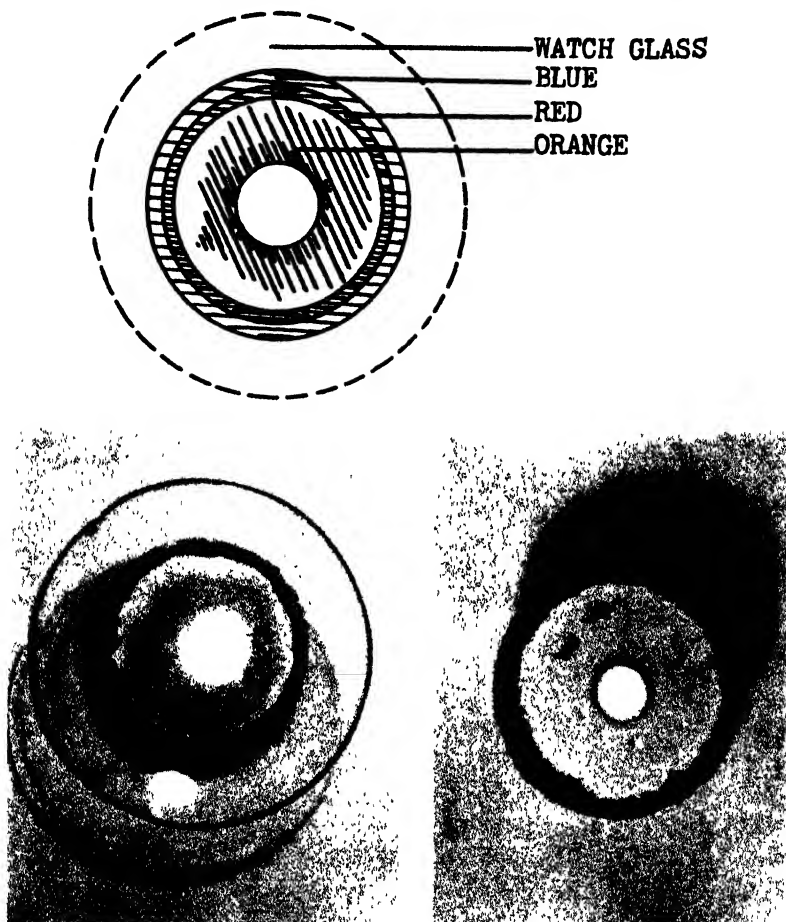


Fig. 1. A Chromatogram on Talc of Methylene Blue, Safranin and Methyl Orange. Left: Upper side; Right: Lower side. About original size.

Mixtures of similarly charged stains can be separated in the same way. A mixture of anilin green, crystal violet and basic fuchsin will divide itself into zones of the above order on talc. The same order

is maintained on magnesia altho the zonation is not distinct. The adsorbed dyes can be removed from the adsorbent by shaking with proper solvent.

The chromatogram of biological stains can also be carried out on a small scale by a much simpler method, which is very convenient for preliminary test and laboratory demonstration. The adsorbent is first put in a small watch glass and then pressed tightly into a compact round smooth cake with a rubber stopper. The dye mixture is then carefully introduced around the circumference of the cake with a capillary tipped pipet. The flow of the liquid should be slow, uniform, continuous and steady. For solutions of moderate concentration, 0.2 or 0.3 cc. is quite adequate. As the dyes are adsorbed



Fig. 2. An Artificial Cell for Illustrating Theory of Staining Nucleus: Talc; Cytoplasm: Magnesia. Stained with Light Green and Basic Fuchsin.

along the margin, they are washed in toward the center with pure solvent so as to differentiate into zones. If correctly done, beautiful color patterns of concentric rings can be obtained on both sides (Fig. 1). In this case, the least adsorbed stain is, of course, the innermost. A variety of adsorbents can be used for the preparation of chromatogram, such as calcium carbonate, starch, etc., most of which are negatively charged like talc.

For the purpose of demonstration of the theory of staining, talc and magnesia are separately mixed with ordinary commercial collodion solution. A portion of the magnesia in collodion is spread on a glass plate and is impregnated here and there with the talc in collodion, corresponding to the nucleus and other chromophilic substances in the cell. The whole cell is then dried and stained as if it

were a histological slide. The parts containing talc take up the nuclear stain (basic) which is contrasted by the magnesia background then colored with the cytoplasm stain (acid). (Fig. 2.)

It is noticed that some mixtures of similarly charged dyes have the same order of appearance on both acid and basic adsorbent. Sudan III is generally known as a fat stain. (See Conn, 1936.) It is neither acid nor basic and does not form salts as ordinary dyes. When a sample of Sudan III (Grubler) is dissolved in alcohol, filtered thru a column of talc or magnesia, and washed with the solvent, two zones can be differentiated: the upper zone is red, while the lower is orange. The two zones can be taken off and redissolved in solution. On both kinds of adsorbents, the order of appearance is the same. Apparently the preferential adsorption is independent of the electrical charge of the adsorbent and behaves just like the components of leaf pigments, the separation of which depends upon the solvent and adsorbent used but the order of appearance always remains the same. It can be readily shown that when the leaf extract is in petroleum ether, pulverized sugar gives the best separation for chlorophyll *b*, chlorophyll *a* and the yellow pigments; when it is in ether or acetone, the components do not separate on sugar, only incompletely on talc but show distinct zones arranged in the same order as before on magnesia.

The third type of preferential adsorption is exemplified by preparing chromatograms of a mixture of methylene blue (basic) and erythrosin or safranin (acid) in alcohol on talc and magnesia separately. Since methylene blue and erythrosin are oppositely charged, one would expect a reverse in order in the two cases. But, as a matter of fact, the zones remain in the same order on magnesia as on talc. The reason for this anomaly is not clear. It is probably due to the formation of an adsorption complex. Magnesium in the form of hydroxide, as reported by Eegriwe (1929), adsorbs numerous dyes and is able to take them up with change of color. It has been observed that by shaking magnesia with a dilute aqueous solution of methylene blue, the stain changes its color from blue to pink on standing.

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NOTES ON TECHNIC

THE USE OF PYRIDINE-FORMALIN IN ZENKER-FORMOL FIXATIVES.¹
—Burke² recommended the use of 5 parts of pyridine to 100 parts of 25% formalin solution to counteract the acidity which develops in commercial formalin. This amount did not interfere with staining by any of the methods tried and greatly improved the silver stains. The salts which he tested were considerably inferior to the pyridine as neutralizing agents because they did not maintain the pH after tissues were placed in the fixative. Pyridine-formalin was suggested for use wherever neutral formalin was specified.

The formol modifications of Zenker's fluid often call for neutral formalin, and the pH of the mixture may be as much as two units higher if the formalin is neutralized with $MgCO_3$ or $CaCO_3$. In this laboratory when pyridine-formalin was used, a heavy precipitate formed and could not be dissolved in ordinary solvents. Sufficient pyridine remained in the blocks of tissue after 24 hours washing to form a hard precipitate at the surface when the blocks were placed in Zenker's fluid. This precipitate was not dissolved by water, alcohol, iodized alcohol, iodine-potassium iodide, ammonium chloride, or dioxan. Mercuric chloride seems to be the substance which causes the precipitate with pyridine but is not itself the precipitate.

It is, therefore, concluded that pyridine-formalin should not be used in fixing mixtures such as Zenker's with or without acetic acid, and it should probably be avoided in any combination with mercuric chloride. Pyridine-formalin can be added to potassium bichromate to make Regaud's fluid, but this seems to have no special advantage.
—VIRGENE WARBRITTON, University of Missouri, Columbia, Mo.

A NOTE ON THE TRESS MODIFICATION OF THE CRESYL VIOLET TECHNIC FOR STAINING NERVE CELLS.—In 1935, Tress and Tress³ presented in this journal a valuable modification of the cresyl violet staining technic. The essential feature was the use of a chloroform-ether-alcohol solution as a differentiating medium.

¹Contribution from the Department of Animal Husbandry, Missouri Agricultural Experiment Station Journal Series No. 505. United States Department of Agriculture cooperating.

²Burke, F. V. 1933. The pH of formalin—a factor in fixation. *Amer. J. Path.*, 9, 915–20.

³Tress, Grace and Tress, Mildred. 1935. A modification of the cresyl violet technic for staining nerve cells. *Stain Techn.*, 10, 105–6.

With this method one may obtain excellent preparations in which the nerve cell bodies are uniformly stained and in which the intercellular substance is almost colorless. We have found, however, that it is almost imperative to use a technical or commercial grade of chloroform in making up the chloroform-ether-alcohol solution. Repeated efforts to obtain well differentiated sections by employing chemically pure chloroform in the differentiating solution led to rather unsatisfactory results. After a number of experiments we have concluded that the presence of a chlorine impurity in technical grades of chloroform is in a large measure responsible for the removal of background stain.

It may be that free chlorine in technical chloroform acts as a bleaching agent in removing stain from the intercellular spaces and from the regions occupied by fiber tracts. Excellent results may be obtained by adding a small amount of chlorine solution to chemically pure chloroform and using this mixture in making a chloroform-ether-alcohol solution. By adding hydrogen peroxide to a chloroform-ether-alcohol solution, one may likewise obtain fairly good differentiation.

Manufactured lots of 'technical' or 'commercial' grades of chloroform contain free chlorine, and the percentage of this impurity in different lots may vary from time to time. Better results may be obtained with one technical grade of chloroform than another. We have found, for instance, that Mallinckrodt's technical chloroform is preferable to Merck's technical chloroform. The chlorine content of chloroform may be increased by passing generated chlorine gas thru this liquid for a short time. Solution of the gas takes place readily, and a strongly saturated solution may be used as a stock reagent in making up more dilute chlorine-chloroform solutions. Experience will indicate the optimum amount of such a stock solution that should be added to ordinary chloroform.

Tress and Tress have also suggested the use of alcohol acidified with very dilute hydrochloric acid in completing the differentiation of cresyl violet stained sections. We have found that an acetic acid alcohol solution (5 to 8 drops of glacial acetic acid to 100 cc. of 95% alcohol) gives more nearly uniform results and a cell stain that is somewhat more brilliant. After passing the sections thru the successive alcohols, they may again be placed in a chloroform-ether-alcohol solution before going into xylene. The use of butyl alcohol is thus made unnecessary.—RALPH W. BARRIS and WM. H. WALLER, Department of Anatomy, George Washington University, Washington, D. C.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

MICROSCOPE AND OTHER APPARATUS

BRIDGES, C. B. An oil-retaining beveled face for high-aperture condensers. *Science*, 84, 335-6. 1936.

Full exploitation of the potential performance of an achromatic sub-stage condenser should include its use with immersion oil, whereby a layer of oil connects it with the object. Not only glare and haze are thus eliminated, but contrast and brilliance of the image are appreciably enhanced. Advantage can then be taken of the maximum N. A. of the optical system for direct observation and photography. The upper face of present condensers is a flat surface. It is suggested that it be beveled off at an angle of about 8 degrees. This slope maintains the connecting layer of oil intact to a surprising degree and eliminates collection of bubbles or air pockets. In filing, a ring of metal of about 0.5 mm. should be left intact around the top lens.—*J. A. de Tomasi*.

MICROTECHNIC IN GENERAL

CARPENTER, E. Another carborundum pencil. *Science*, 85, 226. 1937.

The author's method of making a slide pencil consists in selecting a 4-inch piece of glass tubing suitable to fit an engraver's carborundum point. Cover the blunt end of the latter thinly with sealing wax and while hot insert half way into one end of the tube. Fire polish the other end and introduce card with owner's name.—*J. A. de Tomasi*.

CHEN, T. T. A method for preserving and shipping smears of protozoa. *J. Parasitology*, 23, 112-3. 1937.

Smears are fixed, washed, and run up to 70% alcohol. They are then transferred to vials having an inside diameter of 24 mm., height of 50 mm., and a capacity of 20-25 smears. Place 2 paper rings at bottom of vial partially filled with 70% alcohol. Use paper rings having an outside diameter of 22 mm., inside diameter of 19 mm., and made of fairly thick cardboard. Place one smeared cover slip (No. 1, 22 mm. diameter, round), smeared side down, onto the 2 rings. Insert another paper ring in the vial followed by the second smeared cover slip, with smeared side down. Process is repeated until all smeared covers from a single host have been packed into the vial, which is then filled with 70% alcohol. Place a label bearing the name of the host and locality inside the vial. A wad of cotton is then added which prevents displacement of the contents in transportation. Cork vial tightly and seal with paraffin.—*Elizabeth Bachelis*.

HUBER, P. Die Nuklealreaktion nach Feulgen. *Mikrokosmos*, 30, 116-9. 1937.

The principle of this reaction rests on a partial hydrolysis of the preparation in N HCl and subsequent treatment with fuchsin-sulfurous acid. The nucleins of the cell are composed of an albumin fraction and a nucleic acid fraction. Certain nucleic acids when hydrolyzed split, liberating reducing groups which react vigorously with fuchsin-sulfurous acid, producing an intensive, blue-red color. This re-

action may be carried out in sections, smears, pollen tube cultures, thin membranes, etc. Almost all fixing agents are suitable except formalin and those containing formalin. Deparaffinized sections should remain in alcohol for a few hours. Feulgen recommends the addition of 1% dimedin (Kahlbaum) to bind any aldehydes that may be present. The author finds this unnecessary. The technic followed is that described by Feulgen and Rossenbeck.—*J. M. Thuringer.*

INKSTER, R. G. The use of "cellosolve" for rapid dehydration in paraffin embedding and in the staining of sections. *J. Path. & Bact.*, **44**, 269-72. 1937.

Cellosolve (ethylene glycol mono-ethyl ether) is a good substitute for alcohol for the purposes mentioned in the title. It requires less time and is less hygroscopic than alcohol. For formalin fixed tissues, e.g., cut about 5 mm. thick. Immerse in normal saline 10 min. to remove excess formalin. Transfer directly to cellosolve for 30 min.; 30-60 min.; and 30-60 min., respectively, and to fresh cellosolve $1\frac{1}{2}$ hr. Clear 30 min. in used xylene or 1 hr. in fresh xylene. Embed in paraffin 30 min., and 1 hr., and in fresh paraffin 1 hr. Tissues may be stored in cellosolve without damage. After cutting and mounting, stain sections as follows: xylene, 2 min.; cellosolve, $1\frac{1}{2}$ min.; rinse in water; stain in Delafield's hematoxylin, 2 min.; rinse in 0.5% HCl if necessary; blue in tap water, 15 min.; rinse in dist. water, 1 min. Counterstain with 0.5% eosin in cellosolve, 5 min.; wipe off excess. Clove oil, 30-60 sec.; xylene; Canada balsam.

Cellosolve removes azocarmine very slowly; methylene blue slightly after 1 hr.; eosin fairly rapidly; and neutral red immediately. Hematoxylin is unaffected.—*S. H. Hutner.*

MADGE, M. A. P. The use of agar in embedding small or slender objects. *Ann. Bot.*, **50**, 677. 1936.

Agar can conveniently be used to orient objects for cutting and to facilitate the handling of very small objects, either before or after fixation. When small objects, such as pollen grains or desmids, are being used, the fresh material can be introduced into the agar and fixed immediately after it sets. There is, however, a danger that melted agar may injure delicate objects. The material is fixed, washed and passed up the alcohols, and then transferred to the agar from 70% alcohol, because it can be introduced into the agar more successfully at this stage. The method has been found invaluable for embedding the long styles of *Hedychium* so they may be cut longitudinally, and for embedding delicate root tips to keep them from curling.

Method: (a) A 3% solution of melted agar is poured on a warm slide to form a film 2.5 mm. thick. The objects to be embedded are introduced quickly into this film and oriented. (b) After the agar sets, the film is cut into rectangular blocks containing the objects to be embedded. (c) These agar blocks are easily handled and can be fixed, washed and embedded as if they were the objects themselves, the agar making no difference in the technic used, except that chloroform rather than xylene should be used for clearing and infiltration.

Before cutting, the blocks are trimmed so that the agar is surrounded by a portion of paraffin. It then cuts very easily, and the agar, which remains on the slide after the paraffin has dissolved, does not affect the subsequent treatment and helps to fix small objects to the slide during staining processes. The agar is quite transparent but becomes faintly stained with acidic dyes.—*Elizabeth F. Genung.*

SKILES, B. F. and GEORGI, C. E. The use of synthetic resins in the preparation of permanent bacterial mounts. *Science*, **85**, 367-8. 1937.

Synthetic resins are suggested in place of Canada balsam as mounting media for bacteria. Butyl acetate, free from acetic acid, is the most desirable organic solvent. Resin mounts compare favorably with balsam mounts. Two commercial resins are used: Vinylite (Carbide and Carbon Chem. Corp., New York City) and Pontalite (du Pont de Nemours & Co., Wilmington, Del.). A 15-20% solution is applied the same way as balsam or by flooding the slide tilted at 45°. Drain the excess and air dry for 30 min., or bake at 135° C. for 5 min. without cover slip. For cleaning off cedar oil, use ligroin or gasoline instead of xylene. Mineral oil is a good substitute immersion medium and is much easier to remove. Scratched

surfaces of resin mounts are renewed by covering with another film of the resin. Small organisms (e.g. molds) are easily mounted by sticking them to a thickened layer of resin.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

CANON, H. G. A new biological stain for general purposes. *Nature*, Mar. 27, p. 549. 1937.

Chlorazol Black E "Biological quality" stains nuclei and chromosomes black and cytoplasm and secreted products gray. It can be used for whole tissues as well as sections. Chitin stains green and glycogen, red. The stain requires no mordant or differentiation; terpineol or dilute "Milton" can be used if the sections should be overstained. Chlorazol can be used in either alcoholic or aqueous solutions. A saturated solution in 70% alcohol stains ordinary sections in from 15–30 min. The stain does not fade.

The dye can be obtained from any of the sales offices of Imperial Chemical Industries, Ltd.—*H. D. Reed.*

HOLLBORN, K. Zwei neue Simultanfarbstoffe (Vereinfachte Karmin-Säuregrün-Elastin-"H"-Färbung und verbesserte Gram-Simultanfärbung). *Zent. Bakt.*, I Abt. Orig., 136, 506–8. 1936.

Two new stain mixtures are presented:

(1) Nucplastin (consisting of carmine, acid green and elastin "H"). Dissolve 4 g. in hot soln. consisting of dist. water, 40 cc.; glycerin, 10 cc.; 95% alcohol, 50 cc. Cool and filter. Deparaffinized sections mounted with albumin are transferred from 95% alcohol to stain in "Giemsa" dishes, section downward, for 30 min., rinsed in 95% alcohol, abs. alcohol thru xylene to balsam. Filter each time before using. Results: Nuclei, red; muscle, pink; elastic fibers, brown; dead tissue, yellowish green.

(2) Gram-Simultanfarbstoff "II" (composed of Victoria blue and pyronin). Dissolve 1 g. in 95% alcohol, 50 cc., and 50 cc. glycerin in water bath. Cool and filter. Dilute 1 cc. of stock soln. with alcohol, 1 cc. and dist. water, 1 cc. Stain deparaffinized sections 5–10 min. Rinse in alcohol, clear in xylene to neutral balsam. Smears are fixed in usual manner and may be stained with equally good results. Gram-positive organisms, blue; Gram-negative organisms, red.—*J. M. Thuringer.*

MOORE, J. W. and KINSMAN, J. M. Studies on the circulation: The dye injection method. *J. Lab. & Clin. Med.*, 22, 165–72. 1936.

The dynamics of blood circulation is studied by oral administration of digitalis. To control the action of the drug, use is made of the dye injection method. While the patient rests in the recumbent position, 4 cc. of an aqueous solution of 300 mg. of brilliant vital red are injected intravenously into the upraised arm. The femoral artery is punctured and continuous blood samples are collected in glass tubes at fixed intervals. By this time it is possible to determine simultaneously the velocity of blood flow, flow per minute, total circulating blood volume, volume of blood circulating in lungs and heart, cell volume, and specific gravity of the plasma.—*J. A. de Tomasi.*

ANIMAL MICROTECHNIC

BALEY, J. H. Staining methods for the islets of Langerhans. *J. Path. & Bact.*, 44, 272–6. 1937.

Relatively simple and reliable methods are presented for demonstrating granules in the pancreas. Fresh or formalin fixed pieces of pancreas 3 mm. or more thick should be used; granules have been preserved in pancreas fixed 72 hr. post mortem. One fixing and 5 staining methods are given. Dyes from different sources gave equally good results.

Fix 24 hr. in the following: 3% aq. solution $K_2Cr_2O_7$, 45 parts; 3% aq. solution $HgCl_2$, 45 parts; formalin just before use, 10 parts. Wash in running tap water 24 hr., dehydrate, embed.

Prepare 3 neutral dye solutions by mixing sat. solution of basic fuchsin and methylene blue; basic fuchsin and orange G; and azo fuchsin and gentian violet; filter; wash until the color of the basic dye in each combination predominates; dissolve in abs. alcohol; filter just before using.

1. *Fuchsin-orange method.* Treat thin sections as follows: (1) xylene, 2 min. (2) abs. alcohol, 1 min. (3) Lugol's iodine, 2 min. (4) wash in abs. alcohol, (5) Ehrlich's acid hematoxylin, 20 min. (6) running tap water, 5 min. (7) acid alcohol, until pink color develops (8) wash in tap water, (9) hot mixture of equal parts fuchsin-orange G solution and dist. water, 1 min. on slide (10) blot and rinse quickly in abs. alcohol (11) Lugol's iodine until red color forms, 10 sec. (12) blot (13) abs. alcohol, 2-5 min. (14) xylene (15) Canada balsam. The colors are as follows: nuclei and connective tissue, blue; red corpuscles, bright red; zymogen granules and "granular cells of the acini", bright red.

2. *Fuchsin-blue method.* Treat thin sections as in Method 1, omitting steps 5-7 and substituting fuchsin-methylene-blue for fuchsin-orange G. The colors are as follows: nuclei and connective tissue, blue; red corpuscles, dark blue; zymogen granules, bluish violet; "granular cells of the acini", red, granular cells in islets (β cells), red; non-granular cells (α cells), pale blue.

3. *Fuchsin-violet method.* Treat thin sections as in Method 1, omitting steps 5-7 and substituting fuchsin-acid-violet for fuchsin-orange-G. The colors are as follows: nuclei and connective tissue, unstained; red corpuscles, dark red; zymogen granules, deep violet; "granular cells of the acini", reddish violet; β cells, violet; α cells, unstained. A counterstain of dilute aq. solution of pyronin, neutral red or fuchsin may be used.

4. *Orange-blue-resorcin method.* Prepare stain as follows: Soluble blue extra (Revector from Vector Mfg. Co., London), 1.5 g.; dist. water, 400 cc.; orange G, 2.5 g.; glacial acetic acid, 2 cc.; resorcin, 3 g.; filter after 24 hr. Treat thin sections as follows: steps 1-4 of Method 1; 1% aq. eosin, 10 min.; blot; 1% acetic acid, 2 min.; staining solution, 1 min.; steps 10-16 of Method 1. The colors are as follows: nuclei and connective tissue, blue; red corpuscles, orange; zymogen granules, bright red; "granular cells of the acini", dark red; β cells, red; α cells, blue.

5. *Magenta-blue method.* Treat thin sections as follows: Steps 1-4 of Method 1; flood with boiling acid fuchsin (conc. not given), cool 30 min.; blot; wash in abs. alcohol; rinse in Lugol's iodine; blot and wash in abs. alcohol; Loeffler's methylene blue, 1 min.; steps 12-15 of Method 1. The colors are the same as for Method 1, but fade badly.—V. Warbritton.

BELKIN, M. and SHEAR, M. J. **Chemical studies on tumor tissue. IV. The staining with neutral red of fresh preparations of mouse tumor cells.** *Amer. J. Cancer*, 29, 483-98. 1937.

For the purpose of this study vital staining was selected as the most suitable criterion of viability of tumor cells. The tumor tissue, finely minced, is immersed for a few minutes in a solution of neutral red containing 155 milli-equivalents of sodium chloride per liter. Observations on mouse sarcoma revealed chiefly 2 types of cells: a spindle cell, mostly unstained, with heavy walled nucleus and several nucleoli; and a round cell with a higher affinity for the dye.—J. A. de Tomasi.

BLOCH, F. and GODIN, M. R. **Technique de coloration du foie sur coupes à la paraffine pour le diagnostic histologique de la fièvre jaune.** *Bull. d'Histol. Appl.*, 13, 343-5. 1936.

This technic is a variation of Masson's hemalum-eosin-saffron method for yellow fever lesions in the liver. Fix tissue fragments in formol, pass 24 hr. thru Duboscq-Brasil liquid, embed in paraffin, cut $5\ \mu$ thick, run down to water. Stain 10 min. in Mayer's hemalum (prepared according to Masson), wash in running water and differentiate a few seconds in: 5 drops HCl in 100 cc. 90% alcohol. Immerse 5 min. in tap water to color section blue, stain 3 min. in 1% eosin B (Microcolor) in tap water. Wash thoroly in water. Counterstain 3 min. in alc. saffron extract: soak 2 g. saffron (harvested within a year) in 100 cc. 90% alcohol for 6 hr. at 60° C. Dehydrate in abs. alcohol. Clear and mount.—J. A. de Tomasi.

CROSSMAN, G. **The isolation of muscle nuclei.** *Science*, 85, 250. 1937.

Nuclei of smooth, striated and cardiac muscle are freed from cytoplasm by the following procedure: Smear a slide with Mayer's egg albumen as usual, put 1 drop of 5% citric acid in the center and transfer into it a small portion of fresh muscle.

Gradually the tissue becomes translucent and the acid becomes cloudy from released nuclei; gentle teasing will hasten the process. Remove the muscle and dry almost completely. Fix in 95% alcohol. Rinse repeatedly in tap water and finally in dist. water. Stain thoroly with Mayer's hemalum. Wash in tap water until blue, and counterstain with eosin. Dehydrate, clear and mount. This procedure yields a permanent preparation of blue stained nuclei, suitable for cytological study.—*J. A. de Tomasi.*

FARRAR, G. E., JR. The concentration of nucleated cells in the bone marrow of the albino rat. *Amer. J. Physiol.*, 117, 662. 1936.

The following modification of Isaac's technic for counting marrow cells is given: Dissect shaft of femur free of muscle and cut off the distal end. With usual red blood cell counting pipet take up marrow to first 0.001 mark; dilute to 1.01 mark with 1% acetic acid. Shake in mechanical shaker for 30 min. Fill counting chamber and count cells in 3 sq. mm., multiply by 10,000. This method approximates the accuracy obtained in counting red blood cells in the peripheral blood.—*C. Smith.*

GIOVANNOLA, A. Energy and food reserves in the development of nematodes. *J. Parasitology*, 22, 207-18. 1936.

The presence of glycogen was determined by employing the Best stain, Lugol iodine solution, and the Bauer staining test. The latter, previously used by Giovannola in the study of glycogen in protozoa, has been successfully employed in the present researches. The technic of the Bauer staining test is as follows: After fixation in Carnoy the slides are kept for some time in a solution of celloidin in alcohol-ether to avoid loss of glycogen in subsequent treatments. They are then passed thru alcohol at 60° C., kept for an hour in a 4% solution of CrO₃ in the dark room, washed in water, placed for 30 min. in a solution of fuchsin (Wermel reagent employed in Feulgen test), and transferred for a few minutes into a solution of sodium bisulphite (the same as employed in Feulgen test). Finally they are stained for a short time with Delafield's hematoxylin for contrast. Glycogen shows a deep carmine red stain.

To distinguish glycogen from some other polysaccharides control slides are placed in a solution of filtered saliva in dist. water for an hour at 37° C. This is used to destroy all the glycogen present by the action of glycolytic enzymes.

Fat is studied by Goodey's technic. Nematodes, collected by capillary pipet into a glass capsule containing a drop of water, are then fixed by pouring over them hot 10% alcohol, containing about 2% glycerin. A drop of Scharlach R or Nile blue made up in the appropriate strength, is then added and the capsule set aside to allow the alcohol to evaporate. The worms are stored in weak glycerin.

In the present investigations good results were obtained with the following staining solution: Sudan III, 40 mg.; brilliant cresyl blue, 10 mg.; abs. alcohol, 15 g. Fats stain orange with Sudan III while brilliant cresyl blue gives a contrast blue stain.

For the study of granules of doubtful nature some nuclear stains have been employed such as methyl violet and the Feulgen test.—*D. P. Glick.*

GLYNN, J. H. The application of the Gram stain to paraffin sections. *Arch. Path.*, 20, 896-9. 1935.

The essential feature of the procedure is the use of basic fuchsin at a pH of 2-3. Good histologic differentiation as well as simultaneous staining of Gram-positive and Gram-negative bacteria is obtained.

The details of the procedure of staining, giving the best results with a variety of tissues, are as follows:

Stain with carbol gentian violet for 2 min. The carbol gentian violet is prepared by triturating in a mortar 1 g. gentian violet with 1 g. phenol crystals. Add 10 cc. abs. alcohol. Dilute this stock 10 times with dist. water. Allow to stand for 48 hrs. and filter before using. Drain, but do not wash. Apply iodine (I₂, KI, H₂O, 1:2:300) for 1 min. Apply acetone until no more color is removed (10-15 sec.). Wash in water. Do not allow section to dry. Apply 0.05% basic fuchsin in N/500 HCl for 3 min. This is the essential part of the whole

procedure, and the important point is to have the solution at a pH between 2-3. Drain, but do not wash. Apply sat. aq. trinitrophenol for 30-60 sec. Wash in water. Differentiate and dehydrate in acetone for 10-15 sec. Clear in xylene. Mount in balsam.—*Lull G. Montgomery.*

GORDON, H. A precise silver impregnation method for blood cells. *J. Lab. & Clin. Med.*, 22, 294-8. 1936.

Silver impregnation methods have found little application in hematological work. A simple method is described which can be used profitably on blood films, bone marrow smears and "touch preparations" from spleen or lymph nodes. Air dry the slide and fix a few minutes in 10% formalin; wash in water and mordant 10 min. or longer in 2.5% Fe alum. Wash thoroly in 4 changes of dist. water. Dip in 1% gelatin (to 25 cc. gelatin add 1 drop 2% Na_2CO_3) and drain. Wash quickly in dist. water. Impregnate 5-15 min. in covered dish with diammonio silver hydroxide solution (diluted 1:1 with dist. water): to 5 cc. of 10.2% AgNO_3 add strong NH_4OH drop by drop until precipitate is dissolved; add 5 cc. of 3.1% NaOH and redissolve precipitate with strong NH_4OH ; dilute to 100 cc. with dist. water. Wash in dist. water at 60° C. Reduce a few seconds in alum-formol (10% formalin, 90 cc., 2.5% Fe alum, 10 cc.). Wash thoroly in tap water, dehydrate, clear and mount. All cell patterns, nuclear and cytoplasmic granules appear sharply defined.—*J. A. de Tomasi.*

HOLBERT, P. E. A simple method for fixing and staining spermatozoa. *J. Lab. & Clin. Med.*, 22, 320. 1936.

In the appraisal of semen, an undistorted picture of the morphology of the spermatozoa is essential. The following technic is found to be satisfactory. Allow specimen to become liquid by standing. Make a thin smear as with blood and air dry. Flame gently. Flood slide 2 min. with 0.5% chlorozane, wash gently with running water. Flood and dehydrate 1 min. in 95% alcohol. Drain and allow to dry. Stain 3 min. with 0.5% aq. gentian violet. Rinse in water, 95% alcohol, and again in water. Counterstain 1 min. with 1% aq. rose bengal. Wash in water and dry.—*J. A. de Tomasi.*

HUMPHREY, A. A. A new rapid method for frozen section diagnosis. *J. Lab. & Clin. Med.*, 22, 198-9. 1936.

Where Terry's polychrome methylene blue method is used for a quick diagnosis of tissue biopsies, it is a common experience that fats obscure the picture and alter the staining properties of the dye. The following method is claimed to be simpler, faster, and more reliable: Float section from the freezing microtome into a dish of water and pass to a slide; wipe off excess of water. Stain with 1 drop of 0.5% brilliant cresyl blue (Coleman and Bell) in saline applied to the center of the section; protect with a coverslip and examine. This technic does not give a dark background, thus it obviates the necessity of washing after staining. Over- or understaining is virtually eliminated and the sections do not dry out quite so easily, while the color differentiation is very similar to that given by polychrome methylene blue.—*J. A. de Tomasi.*

INMAN, V. T. and SAUNDERS, J. B. C. M. The ossification of the human frontal bone. *J. of Anat.*, 71, 383. 1937.

This method of demonstrating ossification was described by Skarda (*J. Tech. Meth.*, 13, 38, 1934); it enables one to follow the progress of ossification in a foetus to term. Fresh foetal material is rapidly dissected after freeing from blood by irrigation via the umbilical vessels. The specimens are hardened in 95% alcohol for 48 hrs., cleared (in darkness) in 1% NaOH and finally permanently mounted in 2% formalin solution. The bone is pure white, the cartilage an opalescent blue; shrinkage is about 2% with negligible distortion. Specimens are mounted intact.

If the material is not fresh, fairly good preparations can be made by dehydrating in abs. alcohol after clearing; the material is run from 50% to abs. alcohol in 5 degree stages. Preparation is then mounted in pure benzol. There is more shrinkage than with fresh material.—*H. D. Reed.*

LISON, L. La coloration vitale des nucléoles dans le tube de Malpighi chez *Forficula auricularia*. *Bull. Classe Sci. Acad. R. Belgique*, Série 5, T. 12, 1189-96. 1936.

The vital staining of nucleoli with certain acid dyes has been observed in the Malpighian tubules of *Forficula auricularia*. Out of 65 acid dyes the following 7 were found to stain the nucleoli vitally: Neolane blue 2R, Neolane red BRE, Neolane rose B, Rigane green B, Neolane green B and Rigane green G. Preparations were made by the Société pour l'Industrie Chimique CIBA at Bale. The first 4 dyes stain the best.

By means of a fine pipet inserted between two abdominal tergites, 0.5-2% solutions of dyes were injected into the hemocoel of the insect. Animals were killed after varying lengths of time and dissected in Ringer solution. The dyes were excreted by the tubules. There was no intracellular concentration of the dye and no part of the nucleus other than the nucleolus was stained either diffusely or selectively. Staining of nucleoli began during the first hour, reached its maximum in 2-3 hr. and persisted for 5 hr. The vitality of the nucleoli after staining was proved by the normal rate of excretion in the tubules.—*Alden B. Dawson*.

MOORTHY, V. N. A simple method of staining and mounting nematode larvae. *J. Parasitology*, 23, 100-2. 1937.

About 5-10 specimens of infected cyclops, containing guinea-worm larvae undergoing developmental changes, are isolated in a drop of dist. water on a perfectly clean slide. The specimens are centered and water drained by means of small bits of filter paper. Add 2-3 drops of fixative solution (HgCl_2 , 0.52 g.; NaCl, 1.04 g.; dist. water, 100 cc.). The cyclops die in the solution in 1-2 min. and the guinea-worms in 20-30 min. The cyclops are carefully dissected and the guinea-worm larvae gently released from the body cavities. While larvae are actively moving in the fixative solution place a thin cover slip (No. '0', size $2'' \times 7\frac{1}{8}''$) on the slide and allow the fixative solution to spread evenly under it. After 3-5 min. gently warm the slide over a spirit lamp until small bubbles of gas appear under the cover slip. Cool and examine under microscope. Note position of larvae. Add a few drops of stain on side nearest to larvae (sat. alc. soln. of methylene blue diluted twice with dist. water, 2 cc.; Giemsa stain, conc. stock soln. from Kasauli, 12 cc.; liquor ammonia fortis, 1 drop; dist. water, 50 cc.). Absorb fixative solution from under cover slip by means of filter paper. Add a few more drops of stain at one end of cover slip and keep slide under Petri dish for 24 hr. (For *Paracamelanus*, 12 hr. is sufficient.) If overstained, decolorize with 0.2% HCl. Examine under microscope. Add a few drops of 2% formalin solution on side nearest to larvae. Drain away stain with bits of filter paper. Stained larvae are mounted in 2% formalin solution. Seal cover slips on all sides with gold size. After this is dry seal with Apathy's cement, then apply another coat of gold size.—*Elizabeth Bachelis*.

NAGLE, N. and PFAU, C. L. A modification of Van Gieson's stain for Negri bodies. *Amer. J. Pub. Health*, 27, 356. 1937.

The Williams modification of Van Gieson's stain for Negri bodies in brain tissue gives poor differentiation in certain specimens. The pH of the water used in making up the staining solution is a factor influencing its efficiency. A buffered solution of pH 7.4 gave more uniform staining reactions. The following formula gave good results: 8% alc. solution basic fuchsin, 3 drops; sat. aq. solution methylene blue, 2 drops; buffered dist. water, pH 7.4, 30.0 cc. The diluted stain is very unstable and should be prepared as needed. The stock solutions will keep indefinitely. Technique: fix impression smears with methyl alcohol for 2 min.; wash with tap water; add stain to slide, heat gently to steaming and allow to remain 5 min. without further heating; wash with tap water and blot dry immediately.—*M. W. Jennison*.

OAKLEY, C. L. Frozen sections of eyes. *J. Path. & Bact.*, 44, 365-8. 1937.

The following method is suitable for avian and mammalian eyes: Fix in neutral 10% saline formalin 4 days. Soak eye in frequently changed Muller's fluid for 6 weeks in incubator. Omit post-chroming when haste is desired. Eyes may

be hardened with Perdrau's fluid ($K_2Cr_2O_7$, 5 g.; CrF_3 , 2.5 g.; water to 100 cc.). This may be diluted to half strength. Use at room temp. for 4 days. In large eyes, windows should be cut to hasten penetration, preferably by slicing away sclera until retina is reached, then cutting a hole in the retina. Wash thoroly in running water for at least 24 hr. Cut eye in half, cutting thru optic nerve where the retina is most firmly attached. Soak in 12.5% gelatin overnight, then in 25% gelatin 24 hr. in incubator at 37°. Allow at least 25 cc. gelatin soln. to each half eye. The 25% gelatin cannot be used more than twice. One per cent phenol should be added as preservative. Block, with cut surface downward, in 25% gelatin. Allow to set overnight in a cooled vessel (cold running water will do for cooling; refrigerator is too cold). Cut out blocks, removing superfluous gelatin. Harden in 10% formalin, using a large volume of solution, for 2-3 days and store in 4% formalin. Before cutting soak 15 min. in tap water. Cut frozen sections. Freezing should be slow, until block appears homogeneous. Over-freezing and thawing with water is useful. Weigert's iron hematoxylin differentiated thoroly with acid alcohol and followed by dilute eosin gives good results. Fat staining can be used. Mount in either glycerol jelly or Farrant's medium. Sections will stand alcohol up to 70%.—S. H. Hutner.

ORR, J. W. The results of vital staining with phenol red during the progress of carcinogenesis in mice treated with tar, dibenzanthracene, and benzpyrene. *J. Path. & Bact.*, 44, 19-29. 1937.

In order to determine whether impairment of skin circulation was involved in skin carcinogenesis from chemicals, phenol red (exhibiting its alkaline color in blood but near enough its turning point to be changed to the acid color by accumulating acid metabolites of cells after prolonged contact) was injected into white mice painted weekly for 4-7 months interscapularly with the carcinogens. Injections of 0.5 cc. of a 4% aq. solution of phenol red (Brit. Drug Houses) at pH 7.2-7.9 were given 1 day before the weekly treatment with the carcinogen. Foci of the yellow acid color appeared in the majority of painted areas in which the warts developed; but if the yellow color persisted, the warts developed very slowly or underwent spontaneous retrogression. This was interpreted to mean that the yellow color was due to local, functional ischemia and that conditions favorable to the initiation of lesions might not be favorable to their progress.—V. Warbritton.

SHEAR, M. J. and BELKIN, M. Chemical studies on tumor tissue. V. The staining with vital dyes of mouse tumor cells swollen in salt solutions. *Amer. J. Cancer*, 29, 499-502. 1937.

This paper is a continuation of the work covered by contribution IV by the same authors. Here vital staining and assumed viability of tumor cells are tentatively correlated to swelling of the same cells when in contact with salt solutions which are either hypotonic to the concentration of blood plasma or in other cases, iso-, or hypertonic. It is found that cells, which appear to be of neutral red, manifest enormous swelling, which is apparently not a post-mortem change. Comparable results are also shown by methylene blue and trypan blue.—J. A. de Tomasi.

WANSTROM, R. C. Rapid methods for preparing paraffin sections of tissues. *Amer. J. Clin. Path.*, 7, 78-84. 1937.

The writer gives directions for use in pathological laboratories of hospitals. Paraffin sections are often preferable to frozen sections and rapid methods are available. Photographs of sections fixed 1-2½ hr. show surprisingly good detail.

1. *A routine overnight method.* Treat pieces 8 mm. or more thick as follows: 10% formalin, 1-12 hr. depending on time of collection; 96% alcohol or used abs. alcohol (time not specified); abs. alcohol, 2-4 changes of 1-5 hr. each; xylene, 1 change, 30-60 min.; paraffin, 2 changes, 1 hr. and 6 hr. to overnight; embed.

2. *Intermediate paraffin methods.* Treat pieces somewhat thinner than 8 mm. as follows: 10% formalin, in oven, 15-30 min.; abs. alcohol, 3 changes, in oven, 1-1½ hr.; xylene, in oven, 20 min. and 40 min.; paraffin, 20 min. and 40 min. As an alternate method, treat similar pieces as follows: 10% formalin, 15-30 min.; water-free acetone, 2 changes in 20-40 min.; paraffin, 20 and 40 min.

3. *Rapid method.* Treat small, soft pieces as follows: 10% formalin during transport from operation; abs. alcohol, in oven, 2 changes in 20 min.; xylene, in oven 2 changes in 20 min.; paraffin, 2 changes in 20 min. Instead of the second xylene, a xylene-paraffin mixture may be used.—*V. Warbritton.*

WHARTON, L. R. A technique for studying the innervation of organs. *Anat. Record*, 67, 469-75. 1937.

This is a method of fixing and staining sheets of tissues or thin organs and making fine dissections of nerve plexuses which are especially well stained. Whole mounts in Canada balsam may be made. Method: Tissues must be moderately fresh. Thick organs are not suitable. Tissues are laid on writing paper and allowed to adhere 5-10 min. and are then placed for 18 hr. in the following solution (A): Glycerin, 1 vol.; glacial acetic acid, 1 vol.; 1% aq. chloral hydrate solution, 6 vols. Tissues are then transferred for 24 hr. or longer (until stained a deep purplish blue) to the following solution (B): Glycerin, 1 vol.; Ehrlich's hematoxylin, 1 vol.; 1% aq. chloral hydrate solution, 6 vols.

Proper staining may be tested under the microscope. If overstained, they are decolorized in 70% alcohol containing 1% HCl or returned into solution A. Acid should be neutralized with Li_2CO_3 before preserving the tissues. If understained, tissues may be returned to Solution B. Stained tissues are transferred to glycerin for 10 days and then into fresh glycerin where they may remain indefinitely. Dissections are best made with transmitted light under a binocular microscope giving 5-25 diameters magnification. The specimens are pinned to a layer of paraffin in glass dishes for dissection. For making permanent mounts, proceed from glycerin as follows:

Run tissue thru 70, 80 and 95% alcohol to remove glycerin. Clear in: Bergamot oil, 2 parts; oil of cedar, 2 parts; pure carbolic acid crystals, liquefied by heat, 1 part. Xylene. Mount in Canada balsam.—*S. I. Kornhauser.*

PLANT MICROTECHNIC

BLAYDES, G. W. Preserving the natural color of green plants. *Science*, 85, 126-7. 1937.

The author suggests the addition of 0.2% CuSO_4 to either of the two following solutions: (1) Formalin, 5 cc.; glacial acetic acid, 5 cc.; 50% ethyl alcohol, 90 cc. (2) Formalin, 10 cc.; glacial acetic acid, 5 cc.; 70% ethyl alcohol, 85 cc. The use of these preservatives results in an almost normal green color in nearly all chlorophyll-bearing plant organs. The cellular structure is preserved and the color fixation does not interfere with staining.—*J. A. de Tomasi.*

MADGE, M. A. P. Division of the generative cell in *Hedychium gardnerianum*. *La Cellule*, 45, 171-6. 1936.

Long styles in which pollen tubes are to be followed are treated as follows. Lay the style (already fixed and run up to 70% alcohol) on a slide wet with alcohol and cut into 8 mm. pieces. To a warm slide add a 2.5 mm. layer of warm 3% agar; then lay pieces of style parallel and in proper order in the agar layer. After the agar sets, cut out a square block containing pieces of style, dehydrate in alcohol and alcohol-chloroform series and embed in paraffin. Section and stain with iodine-gentian-violet.—*L. W. Sharp.*

MICROÖRGANISMS

D'ANTONI, J. S. Standardization of the iodine stain for wet preparations of intestinal protozoa. *Amer. J. Trop. Med.*, 17, 79-84. 1937.

A method is described for preparing an accurate iodine stain to be used on wet fecal smears containing intestinal protozoa. It is based on the use of a standardized 1% KI solution to 100 cc. of which 1.5 g. powdered I_2 crystals are added. Allow to stand 4 days and filter. The material is smeared onto a slide by mixing thoroly with 2 drops of saline, using a wooden applicator. Add 1 drop of the stain to half of the smear. Apply cover slips to both halves and examine. (Material can also be concentrated as follows: suspend in an equal volume of water, filter thru cheesecloth, centrifuge twice and decant; to one volume of sediment stain add 2 volumes of sediment.) The entire field is stained a light brown color.—*J. A. de Tomasi.*

EBERSPÄCHER, C. Über neue Entfärbungsmittel für die Tuberkelbazillen-färbung. Zentbl. Bakt., I Abt., Orig., 138, 92-9. 1936.

Altho well over 100 staining methods for the tubercle organism have been suggested, the process of decolorization still presents difficulties. The author finds that Dold's solution (40% aq. urea, 10 cc.; abs. alcohol, 90 cc.) is less drastic in its action than acid alcohol, and quite satisfactory. It is changed 3 or 4 times on the slide, rinsing and blotting dry after each change. Many other products were tried in various solutions and concentrations. Several of the common salts (NaCl, $(\text{NH}_4)_2\text{SO}_4$, NH_4Cl), in combination with ethyl alcohol, will decolorize a T.B. smear satisfactorily. Methyl and propyl alcohol also are excellent solvents for some substances.—*J. A. de Tomasi.*

HEGEDÜS, A. Vitale Färbung von auf farbstoffhaltigen Nährböden gewachsenen Bakterien. Zentbl. Bakt., I Abt., Orig., 138, 99-104. 1936.

The purpose of this study was to determine why colonies of certain bacteria grown on media containing dyes show intense coloration. The following organisms were tested with trypanflavin, fuchsin and crystal violet: *B. dysenteriae*, *typhi*, *paratyphi*, *coli*, *faecalis alkaligenes* and several staphylococci. It was found that the individual cells took on the dye and that their position within the colony determined the degree of staining. Motility as well as multiplication of the organism was not hindered by the dye until its concentration was quite appreciable. At this point bacterial threads and involution forms began to appear. The amount of trypanflavin adsorbed by strains of *B. dysenteriae* of various degrees of tolerance was determined as follows: Weigh 18 mm. cover slips on the analytical balance. Collect on them, by carefully sliding over, part of the growth from an agar plate. Weigh quickly, dry at 37° C. to constant weight. Transfer to a centrifuge tube, crush and comminute with a glass rod. On a water bath extract the dye with boiling 90% alcohol under a reflux condenser. Centrifuge to remove the bacteria, replace the hot alcohol with fresh, until extract is clear. Determine the dye concentration in the total extract by comparing with standard solution of trypanflavin in the Dubosque colorimeter. It was shown that the moisture content of such cultures averages 75-80%; that the concentration of the dye on the organisms is higher than in the medium on which grown; that the amount of dye in bacteria decreases roughly with decreasing concentrations in the medium; that bacteria take on relatively more dye from media with low dye concentrations.—*J. A. de Tomasi.*

SALGUES, R. Les propriétés fongicides préventives du bleu de méthylène en pathologie animale. Compt. Rend. Acad. Sci., 204, 721-3. 1937.

A culture of *Monilia albicans* causing a fatal diarrhea when fed to chicks is rendered innocuous if 1 g. methylene blue per 100 g. ration is included in the diet. It is also of value in severe *Trichomonas* infections. The author believes that methylene blue neutralizes fungal toxins. What may be a similar effect was noted for sheep fed on smut-infected sorghum.—*S. H. Hutner.*

WENBICK, D. H. Studies on *Dientamoeba fragilis* (protozoa). I. Observations with special reference to nuclear structure. J. Parasitology, 22, 76-83. 1936.

Dientamoeba fragilis is shown to be neither so rare nor so incapable of persistence in feces outside the host as has heretofore been supposed. The slides studied were stained with Heidenhain's hematoxylin. Most of the smears were fixed in various modifications of Schaudinn's and Bouin's fluids. Hollande's, Zenker's solutions, as well as sublimate-acetic and picro-mercuric fixatives, were also tried. After most of these fixatives the nuclei of the amoebae stained fairly well in Heidenhain's hematoxylin, except when treated with Schaudinn's fluid without acetic, in which case the nuclei failed to stain. Bouin's fluid was found to be especially good in a modification consisting of sat. aq. solution of picric acid, 75 parts; formol, 15 parts; and glacial acetic acid, 10 parts. When 5-20% of glacial acetic was added to Schaudinn's fluid, the subsequent nuclear staining was usually satisfactory. The one-half strength Schaudinn's fluid containing 2-2.5% acetic acid also proved useful. While hemalum, Delafield's hematoxylin, and Mallory's phosphotungstic acid hematoxylin were tested as stains, none of them gave a nuclear image as sharp as that obtained with Heidenhain's hematoxylin.—*D. P. Glick.*

STAIN TECHNOLOGY

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A MODIFIED ROOT TIP SMEAR TECHNIC

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ABSTRACT.—Iron alum, used as a pre-mordant, makes possible the use of smears on materials that do not smear satisfactorily otherwise.

Chromosomes which do not stain well after storage when the usual methods are employed give satisfactory results with the modified method.

In root tip smears of *Citrus*, *Rubus*, and *Zephyranthes texana*, the chromosomes fail to take the stain or stain very poorly in smear preparations prepared by any of the usual smear methods. The method described by Warmke¹ has been found to give good preparations with



Fig. 1. Metaphase plates of Citrange (*Poncirus trifoliata* x *Citrus sinensis*) from root tip smears, using modified method. $\times 1500$. $2N = 18$ chromosomes, plus fragment. Note clearness with which constrictions appear in chromosomes.

The author was unable to obtain any satisfactory preparations from this plant by employing the usual smear methods.

many plants, but does not give satisfactory results with the plants listed above. For this reason a modification of Warmke's method has been developed that allows good preparations (Fig. 1) to be made with material that does not stain well with other methods. This modification, which is based on a pre-staining mordant for its effect, is briefly described below.

¹Warmke, Harry E. 1935. A permanent root tip smear method. *Stain Techn.*, 10, 101-3.

Young root tips are fixed in Carnoy's solution (6 parts absolute alcohol; 3 parts chloroform; 1 part glacial acetic acid) from 12–15 hours. If the material is not to be used immediately following fixation it may be stored in the fixative or 70% alcohol. If storage is to extend over a period of more than three or four days, 70% alcohol is preferable as a storing agent.

When ready to make preparations place the root tips in a solution of 1 part concentrated hydrochloric acid to 1 part 95% alcohol, to which has been added *just before using*, an equal amount of a 4% aqueous solution of iron ammonium sulfate (iron alum). Tips should remain in this solution from 10–15 minutes, depending on their size, after which they are smeared in iron aceto-carmine in the usual manner.

If the desired division stages are found to be present, mounts may be made semi-permanent by sealing with a mixture of paraffin and gum mastic. Preparations of this sort, if kept in the dark, will keep for a period of 10 days to two weeks. If further permanence is desired, McClintock's² permanent method may be used.

The above-described method has also been found to give sharper differentiation than most smear methods when used on root tips of *Allium*, *Triticum*, *Quercus* and *Gilia*.

It is known that some smear methods fail to give satisfactory results when used on root tips that have been in storage over long periods of time. With this in mind, root tips of *Quercus*, *Rubus*, *Allium*, and *Gilia* were stored in 70% alcohol and preparations made at 10 day intervals up to 40 days. From the results obtained, using the modified method described, it seems that the chromosomes take the stain equally as well after a period of 40 days storage as they do immediately following fixation.

²McClintock, Barbara. 1929. A method for making aceto-carmine smears permanent. *Stain Techn.*, 4, 53–6.

A SMEAR TECHNIC FOR DEMONSTRATING CELL INCLUSIONS WITH CHARACTERISTICS OF BOTH MITOCHONDRIA AND BACTERIA

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ABSTRACT.—Cytoplasmic inclusions of various types of cells have been investigated by macerating or smearing and fixing and staining by different mitochondrial methods of technic. The results obtained as regards granular, rod-like, filamentous and globular forms immediately suggest a relation between these and similar cell inclusions which have in the past been described as mitochondria in certain cases of this material. While mechanical disturbance and drying before fixation apparently do not alter the staining properties of these forms, alcohol produces somewhat variable results depending upon the kind of material being investigated. Results indicate the presence in these smears of numerous intracellular bacteria, readily misinterpreted as mitochondria. In addition, there occur in certain cells, both in smears and sections, inclusions of indeterminant nature.

The aim here is to describe the procedure and to give in a brief manner some results of preliminary investigations of certain cell inclusions by means of smears. The different types of cells investigated include both plant and animal. By employing mitochondrial technics, smears have in a number of cases produced somewhat surprising results, in that cell inclusions of a granular, rod-like, filamentous, or globular nature have been frequently observed. The forms occurring in smears bear such a close resemblance in size, morphology, and staining reaction to cell inclusions found in permanent preparations of like material by mitochondrial methods that the question of relationship and significance immediately arises.

Granules and rods have been observed in smears made of *Amoeba proteus*, *Paramecium caudatum*, *Peranema trichophorum*, and *Spirostomum ambiguum* (after washing thru several changes of sterile water and starving for a short period of time) and thoroly macerating on a clean slide in a drop of sterile water, drying, fixing in Altmann's, Champy-Kull's or 2% osmic acid vapor, and staining with anilin fuchsin and methyl green or toluidine blue. The procedure is quite

¹Miller, E. DeWitt. 1937. A comparative study of the contents of the gelatinous accumulations of the culture media and the contents of the cytoplasm of *Amoeba proteus* and *Arcella vulgaris*. J. Morph., 60, 325-53.

simple in the case of *Paramecium*, *Peranema*, and *Spirostomum*, while in the case of *Amoeba* it has been found advantageous, if not necessary, to employ a minimum amount of fluid medium when macerating to demonstrate cytoplasmic inclusions possessing characteristics of the alleged mitochondria and frequently observed in permanent preparations with mitochondrial technics. Among the ciliates mentioned above, rods and granules are frequently numerous. These rods and granules look and stain as one would expect of mitochondria if present. Occasionally smears of *Paramecium* will show granules of this nature only, but rods are present also in the majority of cases. This variation relative to the number of rods or granules present has been observed in sectioned material of this ciliate by the writer and by other workers. Rods present in smears of *Paramecium* can be distinguished from trichocysts by their smaller size, difference in shape, and in their staining reaction.

Similar tests have been carried out on other kinds of materials. These consist of the rhabdocoeles (*Microstomum*, *Macrostomum*, and *Stenostomum*), liver, pancreas, kidney, spleen, and the root nodules of clover. Smears of all these tissues have shown minute intracellular granules, rods or filaments, or combinations of these, and frequently in large numbers. Filaments have occurred most frequently in smears of the pancreas. The staining properties, size and morphology of many of these forms observed in smears are identical with those of granules, rods or filaments occurring in permanent preparations or sections of the same kind of material, and heretofore described as mitochondria in certain of these cases. In some cases comparative studies of smears and sectioned material have shown that inclusions of the above-mentioned type, and characteristic of the particular kind of material, are frequently brought to light in a more satisfactory manner by the smear method.

Smears in a number of instances have been subjected to prolonged treatment with 95% alcohol prior to treatment with a mitochondrial fixative with somewhat varying results. Among those of *Paramecium*, *Peranema*, and *Spirostomum*, alcohol apparently does not alter the staining qualities of the minute inclusions. Somewhat similar results have been observed among smears of *Microstomum* and *Stenostomum*. Among smears of mammalian tissues, liver and pancreas, the results following alcohol treatment have been varied, altho in every case of non-alcohol treated smears which were examined granules, rods or filaments have appeared. In case of liver smears where the studies are more nearly complete, these minute forms have undoubtedly had their staining properties altered, or have sometimes been

destroyed by alcohol, even tho the mode of treatment was in so far as possible identical. The results obtained in the case of liver smears would indicate that among cell inclusions of this nature there exists a difference in resistance to alcohol treatment in different regions of the liver.

As to the true nature of all such small forms occurring in smears, investigations are not as yet sufficiently complete in the majority of cases mentioned to warrant very definite conclusions. Further investigations, however, are now in progress in this laboratory to determine, if possible, the relationship between these and similar inclusions heretofore described as mitochondria in some of this material.

In a recent paper I have concluded that in *Amoeba proteus* and *Arcella vulgaris*, inclusions which have been thought by some workers to represent mitochondria, are possibly symbiotic or commensal bacteria. The results of an investigation just completed on the nodule of the roots of clover indicate that minute bacterial forms from the soil, which are believed generally to enter the cells of the plant rootlet, have been misinterpreted in the past as mitochondria.

In view of the current opinion of previous workers in this field that mitochondria are destroyed by mechanical disturbance (separation from the cell), drying and by lipoid solvents such as alcohol, the results obtained here show that these forms occurring in smears either are not mitochondria or else mitochondria possess properties not as yet fully recognized.

Repeated trials by various methods of technic upon some of this material show convincingly that these forms occurring in smears are not merely artefacts but that many are obviously bacteria of an intracellular nature. In addition these results indicate that bacteria, bacteria-like, or similar morphological cell inclusions have frequently been misinterpreted as mitochondria.

DIOXAN SCHEDULE FOR COMBINATION PLANT-ANIMAL TISSUES¹

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Recent articles by McClung² and by McWhorter and Weier³ in this magazine have emphasized the advisability of using dioxan as a substitute for alcohol in the paraffin method applied to plant tissues. Baird⁴ has discussed alcohol substitutes for embedding animal tissues. In studying the penetration of plant tissues by aphids, I have used with uniformly good results a dioxan schedule differing slightly from those described. Dehydration with dioxan permits rib-



Fig. 1. The aphid *Myzus persicae* feeding on a beet leaf. Note how insect as well as plant parts are preserved. 145X.

bon sections of even woody materials without disturbing the delicate proboscis and stylet structures of the aphids. For example, dioxan embedding has given excellent results with pieces of rose stems up to 6 mm. in diameter and 25 mm. in length.

Plant parts infested with aphids are plunged into chloroform to kill the aphids and transferred immediately to formalin acetic alcohol

¹A more complete paper on the insect phases is being published in the *Journal of Agric. Research*.

²McClung, C. E. 1936. A dioxan technic. *Stain Techn.*, 11, 121-2.

³McWhorter, Frank P. and Weier, Elliot. 1936. Possible uses of dioxan in botanical microtechnic. *Stain Techn.*, 11, 107-17.

⁴Baird, Thelma T. 1936. Comparative study of dehydration. *Stain Techn.*, 11, 13-22.

where they are fixed for 24 hours. On removal from the fixative the tissues are rinsed in dioxan, and dehydrated in dioxan over calcium chloride for from 4–12 hours, the time varying with the size of the pieces. They are then passed thru various mixtures of dioxan and xylene as follows: 1) Dioxan and xylene, 4:1; 2) Dioxan and xylene, 3:2; 3) Dioxan and xylene, 2:3; 4) Dioxan and xylene, 1:4.



Fig. 2. The aphid *Macrosiphum rosae* feeding on a rose stem. Note how insect as well as plant parts are preserved. 130X.

The length of time required in each mixture varies with the size of the pieces and the kind of plant tissue. For thin, entire leaves and stem pieces not over 3 mm. in diameter, 15 minutes in each solution is sufficient. For thicker leaves or larger pieces of stems the time must be increased somewhat, but 25 minutes in each solution has been found satisfactory for pieces as large as it is practicable to embed. Tissues are rinsed in xylene after No. 4 mixture, and then placed in pure xylene. Paraffin is added to the xylene, and embedding proceeds in the usual manner. Xylene is a better paraffin solvent than dioxan and thereby insures more uniform penetration.

The above schedule was developed by the writer in connection with a thesis problem at Oregon State College as a part of the requirements for the degree of doctor of philosophy.

THE APPLICATION OF GLYCHROGEL MOUNTING FOR TREMATODES

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Since the introduction of a gelatin embedding and glychrogel mounting technic for frozen sections by Zwemer,¹ this method has been successfully applied in other fields. For a number of purposes a water-type mounting medium has been found to be of great advantage. The successful use of glychrogel for total mounts of small animals such as nudibranchs by Wotton,² suggested the possibility of its application for certain of the animal parasites. In the present paper, a simple method is described for staining and permanently mounting in glychrogel the common trematode of the frog lung, *Pneumoneces medioplexus*.

Procedure: Specimens of *Pneumoneces medioplexus* were obtained from the lungs of freshly killed frogs (*Rana pipiens*) and immersed in distilled water until most of the eggs had been expelled from the uterus of the worms. This may easily be observed under the dissecting microscope. After fixation in 5–10% formalin for 10–20 minutes, the worms were rinsed several times in distilled water. They were stained in Ehrlich's hematoxylin over night, altho it was found this time could be reduced to an hour or less. They were then destained in 0.2% aqueous solution of hydrochloric acid. The color removal was checked by occasional observations under the microscope. All traces of acid were removed by washing the specimens again in several changes of distilled water, and then with 0.5% solution of ammonia water until they became blue. At this point, after rinsing again in water, they were either mounted directly in a large drop of fluid glychrogel or first immersed in a 5–10% solution of glycerin to aid in clearing. The glycerin solution makes an excellent preservative and the stained trematodes may be stored in it in tightly stoppered bottles away from direct light until such time as they are to be mounted.

Comment: Glychrogel should be warmed in an incubator or water bath prior to use to render it of the fluidity of glycerin jelly. A very

¹Zwemer, R. L. 1933. A method for studying adrenal and other lipoids by a modified gelatin embedding and mounting technique. *Anat. Rec.*, **57**, 41–4.

²Wotton, R. M. 1936. The morphology of the nudibranch, *Stilliger fuscatus*, from Staten Island, New York. *Proc. Staten Island Inst. of Arts & Sciences*, **8**, 8–12.

large drop should be placed on the slide and the specimen arranged before the cover glass is lowered into position. It is important that there be an excess of mounting medium about the edges of the cover, because on hardening glychrogel shrinks and air may be pulled in at the edges. The mount should be relatively stable in 12-24 hours. Altho not necessary, it is advisable to ring the edges of permanent mounts with gold size, Duco paint, or some other such material. The formula for preparation of glychrogel and the optical properties of this mounting medium have been reported elsewhere.³

Delicate specimens subject to injury from shrinkage and distortion during dehydration may be quickly and permanently mounted without danger in glychrogel, and the satisfactory results obtained with *Pneumoneces medioplexus* indicate that this procedure could likewise be employed for other similar parasitological specimens.

³Wotton, R. M. and Zwemer, R. L. 1935. A note on 'glychrogel' mounting solution. Stain Techn., 10, 21-2. See also footnote 1.

THE DIOXAN TECHNIC

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ABSTRACT.—Dioxan is recommended in place of alcohols and clearing oils in paraffin embedding and in the staining of sections. It is unnecessary to dehydrate fresh dioxan before using and the insertion of other dehydrators and clearers into the series is illogical. Used dioxan (if employed for the sake of economy), should be dehydrated with CaO rather than with CaCl₂. A provisional dioxan-iron-hematein method designed to avoid watery solutions is as follows: after removing paraffin in xylene, mordant 30 min. in 1% ferric chloride in 100% dioxan, rinse in 80% dioxan, stain in the following solution: dioxan, 40 cc.; water, 6 cc.; glacial acetic acid, 4 cc.; hematein, 5 g., saturated with potassium alum and filtered. Differentiate in 0.25% picric acid in 80% dioxan and alkalize in 80% dioxan saturated with sodium bicarbonate. Rough determinations of the solubilities of various salts and dyes in dioxan are presented. A summary is given of the unpublished experiences of other workers with a variety of both plant and animal tissues. A brief historical account of the development of the dioxan technic is included. A summary of pharmacological studies indicates that dioxan is not dangerously toxic in concentrations likely to be encountered in the microtechnic laboratory.

INTRODUCTION

The first to use dioxan in microtechnic were Heinz Graupner and Arnold Weissberger of the Zoological Institute and the Chemical Laboratory, respectively, of the University of Leipzig. They published their first paper on the paraffin method in 1931 and a second in 1933 on the freezing method.

The possibility of using dioxan as a clearing agent was first suggested to me in 1933 by Dr. A. H. Uhl (Department of Pharmacy, University of Wisconsin) in reply to a remark that the ideal substance for microscopists would be some liquid mixing freely with both water and paraffin. He suggested dioxan, saying he believed it had the desired miscibilities. Accordingly, a Zenker fixed squirrel ovary and oviduct which were washing in water were transferred directly to dioxan. After two changes they were put in paraffin for about two hours and embedded. A complete series was cut and mounted the same afternoon, and stained and covered in the evening. Even this first attempt gave results equal to those of the routine paraffin

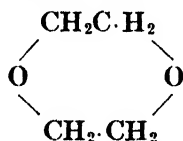
method. In a few days other tissues from several other fixatives, including a Bouin's fixed spadix of skunk cabbage (*Symplocarpus foetidus*) were run thru by this method. All turned out well.

References to the work of Graupner and Weissberger (1931, 1933) were soon noticed and complete translations of both their papers were made; mimeographed copies of these were distributed to several people, both at Wisconsin and elsewhere. In 1934 the method was demonstrated at the meetings of the American Association of Anatomists in Philadelphia. In May 1934 a note in *Turttox News*¹ helped to call it to the attention of biologists generally. Since 1934 the technic has spread in this country, (see bibliography) until there seems to be no longer any doubt of its importance both in zoölogical and botanical work. It is also being used widely in England.

Among those who have contributed most to the development of the method in this country are Johansen (1935) and Baird (1936) who compared the results of dioxan dehydration with those of other dehydrators. Conant (1935) in notes on his experiences with it published from time to time in his "Triarch Topics" has helped call the attention of botanists to it, but the best sources of information on its application to plant work are the papers of Backman (1935) and McWhorter and Weier (1936).

PHYSICAL AND CHEMICAL PROPERTIES OF DIOXAN

Dioxan, (perhaps a better English spelling would be *dioxane*) is 1:4-diethylene dioxide. Its structural formula is:



It is obtainable at a reasonable price from several sources, but that which I have used is the commercial grade, labeled "Dioxan" and marked "passed," sold in gallon cans by the Carbide and Carbon Chemicals Corporation, 30 East 42nd Street, New York City. It should retail for about \$3 per gallon. It is completely miscible in all proportions with water, ethyl alcohol, clearing oils and melted paraffin. It dissolves balsam and gum dammar and slowly dissolves cold paraffin. Dioxan is inflammable, having somewhat lower volatility and about the same flash-point as 95% alcohol. Dioxan vapor in the concentration of 1:1000 parts in air is definitely poisonous, but such concentrations are not likely to occur in any ordinary technic laboratory.

¹Anonymous. 1934. Dioxan. *Turttox News*, 12, 162.

The following table presents for comparison some of the physical constants of ethyl alcohol and dioxan.

	Mol.	Sp. Gr.	M. P.	B. P.	Flash-point		Vapor Pressure 20°C.
					Observed	Calculated	
Ethyl alcohol	46.05	.789	-117.3°C.	78.5°C.	9°-32°C.	14°	43.9 mg. Hg.
Dioxan	88.08	1.0418	8°C.	100.8°C.		11°C.	29.0 mg. Hg.

The high freezing point makes dioxan especially desirable for the frozen section technic. Also the relatively low vapor pressure combined with moderate boiling point and flash-point contribute to its ease of removal in the paraffin bath with relatively low fire hazard.

THE ROUTINE DIOXAN PARAFFIN TECHNIC

A routine technic for a piece of animal tissue 5 mm. thick, fixed in Bouin or alcohol-formol-acetic, is as follows: 100% dioxan (direct from fixative) 1 hr.; 100% dioxan 1 hr.; 100% dioxan 2 hrs.; melted paraffin 15 min.; melted paraffin 45 min.; melted paraffin 2 hrs.; embed in clean paraffin.

Since dioxan is heavier than paraffin, the containers should be shaken before changing paraffins. With delicate tissue it is better to make the transfer from the fixative to dioxan and from dioxan to paraffin by steps, that is mixtures of $\frac{1}{2}$ fixative $\frac{1}{2}$ dioxan, or even by fourths. Also it is better with spongy or hollow tissues such as testes or gestation sacs of pregnant uteri to place the tissues while still in the fixative in a vacuum chamber for 12-24 hr. This is necessary when transferring from an aqueous medium to dioxan because the latter dissolves very little air and bubbles are likely to form in the tissues unless the air has been previously exhausted. A simple apparatus for this is a chemical vacuum dessicating jar attached to an ordinary water vacuum pump. This is not an additional complication of the dioxan technic as this precaution should be taken with such tissues in the ordinary alcohol-clearing oil methods. Used dioxan may be reclaimed satisfactorily for re-use in the first changes by keeping it in a flask with a few lumps of unslaked lime, CaO_2 .

GENERAL DISCUSSION OF THE PROPERTIES OF DIOXAN AND THEIR APPLICATION IN MICROTECHNIC

It is unnecessary to treat new dioxan with calcium chloride or calcium oxide to remove water before using. Commercial samples of dioxan of the type described always mix readily with melted paraffin forming a clear solution. It is said that dioxan has a strong affinity for water and is unstable when exposed to air, yet when left exposed in

an open dish for 24 hr. the reactions with melted paraffin and other chemicals have been unaltered, altho there is slight evidence of water absorption as shown by copper sulfate. Aside from all this, the fact that it works without preliminary dehydration is all that we need to know. Perhaps a trace of water is present, but if there is, it makes no difference in practical use. Crude tests with our samples showed that dioxan containing approximately 0.5% of water will not cause cloudiness when a few drops are added to 3 cc. of melted paraffin, but with 1% water there was cloudiness. It is certainly "good technic", however, to keep any such fluid tightly stoppered.

The belief in the necessity for dehydration of the dioxan started with Weissberger and Graupner (1931) who recommended treatment with anhydrous calcium chloride *during* the process of dehydration or dealcoholization of tissues. Their idea was that the calcium chloride would take the water and alcohol brought in by the tissues and thus keep the solution fresh for continued use. This calcium chloride treatment of the fresh dioxan was tried here, but it was found that if allowed to stand a few days the chloride would swell enormously as if water or alcohol were present in large quantities, altho no tissues were brought into it. A chemist furnished the information that dioxan itself reacts in some way with calcium chloride and, therefore, calcium oxide should be used. This did not disintegrate on standing, but it was soon found that even this treatment was unnecessary. The sensible thing would seem to be to test the dioxan with melted paraffin. If a clear mixture results there is no necessity for preliminary treatment of the dioxan. Used dioxan can best be treated with calcium oxide because it takes out the water and neutralizes acids rendering it entirely suitable for the first one or two changes in the dehydration process.

One property of dioxan of which many do not seem to have made full use is its complete miscibility with melted paraffin. There is seldom any reason, at least with animal tissue, therefore, for using other dehydrating or clearing agents in a series with it. Of course, if a cleared specimen is desired for examination or dissection before embedding, or for a whole mount, some clearing agent such as anilin, winter-green oil or xylene must be used, but there is certainly no logic in interposing alcohols, acetone, chloroform, etc.

Other properties of dioxan which have not been fully utilized are its unusual solvent capacities. For instance, it dissolves large amounts of mercuric chloride, ferric chloride and metallic iodine, while potassium bichromate, iron alum (ammonio-ferric sulfate), sodium bicarbonate and potassium permanganate are practically in-

soluble in it. The addition of 20% or less of water forms a mixture in which all these chemicals dissolve. In dealing with tissues fixed in mixtures containing potassium bichromate, it is necessary to wash well in water before placing them in dioxan in order to avoid crystallization of the bichromate. On the other hand, tissues from fixatives with mercuric chloride may be washed in water to remove the excess, then transferred to dioxan and freed of bichloride crystals; or, if there is a large amount of bichloride, metallic iodine may be dissolved in the dioxan so that in either case dehydration and removal of the bichloride are carried on together without the necessity of long treatment with iodized alcohol.

In general, oil soluble dyes dissolve in dioxan, while those soluble in water and alcohol do not, at least to any great extent. The following results were obtained from crude tests on some of the commonly used dyes.

Insoluble in dioxan: Acid fuchsin, anilin blue, Berlin blue (so-called "soluble Berlin blue" used for injection work), carmine (ordinary), carmine (water soluble product after alcohol precipitation).

Weakly soluble in dioxan (solutions too weak to be of practical use in staining): Eosin Y, erythrosin, Janus green, light green, methylene blue, orange G, safranin, toluidine blue.

Soluble in dioxan: Alizarin (C. I. No. 1034), hematein and hematoxylin (in both neutral, weakly acid, and weakly alkaline solutions, but much more soluble if 1% water is added), picric acid (gives slightly straw-colored solution), Sudan IV.

Soluble in dioxan plus about 1% water: Acid fuchsin, alizarin, anilin blue, eosin Y, erythrosin, Janus green, hematein and hematoxylin (neutral, acid, and alkaline), light green, methylene blue, orange G, safranin, Sudan IV, toluidine blue.

Carmine and Berlin blue remain insoluble until about 50% of water is added. Alizarin and Sudan IV begin to be markedly less soluble after the addition of 2 to 3% of water.

Thus it is possible to substitute dioxan for alcohols in the staining series as well as in the dehydrating and embedding process. The following schedule has been used for over a year:

- 1) Xylene (for removing paraffin); 2) xylene (rinse); 3) 100% dioxan (for removing xylene); 4) 100% dioxan (for removing xylene); 5) Mann's acid hematein (or Delafield's or Ehrlich's hematoxylin); 6) distilled water (rinse); 7) 50% dioxan 50% water, plus 0.1% HCl (destain); 8) 80% dioxan 20% water saturated with sodium bicarbonate (for blueing); 9) 90% dioxan 10% water (rinse); 10) 0.1% erythrosin in 90% dioxan 10% water (counterstain); 11) 90% di-

oxan 10% water (rinse); 12) 100% dioxan; 13) xylene; 14) xylene. After step 11, if one wishes a triple stain, the slide may be transferred to 0.1% orange G in 90% dioxan and 10% water until the orange G has extracted the erythrosin from the connective tissues and stained them yellow and orange in contrast to the muscular tissues and blood which retain characteristic tints of pink and red. The process may be stopped at the proper time by transferring to the 90% and 100% dioxans, the same as after erythrosin or eosin alone. Instead of dioxan acidified with HCl, a weak picric acid solution in the same mixture may be used. This is especially good after iron hematoxylin. In the latter case one should transfer the slides from dioxan to water before placing in the iron alum mordant and, of course, again before staining in the aqueous hematoxylin solution.

Recently hematein stains made up in dioxan containing 20% or less of water have been investigated. After removing the paraffin with xylene and passing into 100% dioxan (step 3 in the preceding schedule) the slides are mordanted for about 30 min. in a solution of 1% ferric chloride in 100% dioxan. They are then rinsed in 80% dioxan (20% water) and placed in a hematein solution made as follows: 2.5 g. of hematein are dissolved in 40 cc. of dioxan to which 8 cc. of distilled water and 2 cc. of glacial acetic acid have been added. After the hematein is thoroly dissolved about 2.5 g. of potassium alum are added and shaken with the solution until it is saturated with the alum. When filtered it is ready for use. Some tissues stain well in this mixture in a few minutes, but differentiation seems to be more easily accomplished and also sharper if staining is carried on for from 30–60 min. The slides are destained in 1% picric in 80% dioxan and rinsed in 80% dioxan saturated with lithium carbonate (step 8 of the previous schedule). This gives a very sharp bluish-black nuclear stain. This solution will stain without mordanting if enough time is given, and probably a much more concentrated solution of the stain would work still more efficiently. Apparently this hematein solution retains its strength for only one or two weeks. There is no particular advantage in the dioxan-hematein method, however, except the avoidance of the more watery solutions. This may have a distinct value in treating sections which tend to become loosened from the slide in aqueous solutions.

Dioxan is of advantage in the Mallory tricolor method where, after staining in Solution II (anilin blue and orange G), the slide may be rinsed in water and destaining stopped immediately by transferring to 100% dioxan, or slow differentiation may be carried on in a mixture of dioxan and water. The stains should not be dissolved in dioxan

mixtures. Waterman (1937) describes a Mallory tricolor method utilizing dioxan in the rinse solution, but not in the stains.

Letters from a dozen or more workers at other institutions have brought further valuable information. It has been shown that dioxan gives excellent results in the preparation of various materials such as vertebrate tissue in general, land snails, insects, frog oviduct, snake tissues (including skin), with special mention of the skin and legs of canary birds from which serial sections are otherwise very difficult to obtain. From the Department of Embryology of the Carnegie Institution at Baltimore, reports have come of excellent results with this reagent in the dehydration of insect salivary gland chromosome smears stained in aceto-carmin and of insect spermatocyte preparations fixed in Gilson's fluid and stained in iron hematoxylin. This same laboratory reports that tissue so hardened by long exposure to 95% alcohol that it cut very poorly by the usual celloidin-paraffin technic, sectioned perfectly after removing from this embedding medium and placing in dioxan (3 changes) for 5 weeks, with subsequent transfer to dioxan-paraffin (48 hours) and embedding in 52° paraffin.

Graupner and Weissberger's (1933) dioxan fixative, as well as many modifications of it, has not been found to give good results on mammalian testis. Modifications of Bouin's and alcohol-formol-acetic with dioxan in varying proportions work fairly well on some of the more stable tissues but they are poor when tested out on a delicate tissue such as testis. Dioxan may be a good fixative for some non-mammalian tissues, but it is of doubtful value for mammalian material.

Tissues may be stored in dioxan without ill effects. Comparison of a fetal pig ovary fixed in alcohol-formol-acetic and stored in dioxan for over two years, with its mate sectioned by the dioxan method at the time the pig was obtained, showed no change in the tissue either structurally or in staining capacity. Two squirrel ovaries fixed in formol-Zenker were stored for nearly three years in dioxan and when recently sectioned stained well and seemed to have suffered no damage. Some tissues may, of course, harden or undergo some change, but so far there is no evidence that they do.

The possibility of using dioxan in the celloidin technic has also been investigated. Cellulose nitrate is practically insoluble in dioxan while cellulose acetate (from "safety" films) dissolves readily. Addition of 10% of acetone makes the nitrate soluble also. No method of satisfactorily hardening a dioxan solution of either the acetate or nitrate has been found, altho a variety of chemicals such

as chloroform, carbon tetrachloride, etc., have been tried. Tissues dehydrated in dioxan may be transferred to ordinary alcohol-ether solutions of cellulose nitrate and embedded successfully.

DIOXAN IN BOTANICAL TECHNIC

Several botanists have published articles on the use of dioxan. Backman (1935) gives a detailed schedule for its use in making cytological preparations. She thinks the dioxan must be thoroly dehydrated and that, altho it does not cause shrinkage, the images in root tips are not as clear as those dehydrated with alcohol. Johansen (1935) recommends both dioxan and tertiary butyl alcohol and believes one of their chief advantages over other dehydrators is that they are not "dessicators", that is, they do not remove the "combined" water. Dufrenoy (1935) recommends methylal, $\text{CH}_2(\text{OCH}_3)_2$, a fluid which seems to be in many physical properties quite similar to dioxan. It mixes with both water and paraffin. McWhorter and Weier (1936) have published the most extensive paper to date on the use of dioxan in botanical technic. They recommend a combination of dioxan and xylene in passing into paraffin, a procedure which appears to be illogical. They have devised a dioxan-containing fixative and also given procedures for a variety of whole mounts. They found that very soft tissues, such as ripening grapes, remained too soft when treated by the dioxan method. They also discuss briefly some of the possibilities of dioxan in staining and in the preparing of mounting media of different refractive indices.

As I am not a botanist, no personal experiences are available along this line, but communications from botanists have been received in the course of the inquiries that have been made as to the experiences of others. The results thus communicated are conflicting, by no means all the botanists obtaining the good results reported by McWhorter and Weier. Plasmolysis and shrinking of the tissues have frequently been noted. Some of the botanists report good results with leaves only when fixation or dehydration is carried out in a partial vacuum.

Summarizing the information at present available it can be said that the dioxan method may not be as universally satisfactory in botanical technic as in animal work. In view of the discrepancies in results of different workers, however, one is inclined to believe that many of the faults attributed to the use of dioxan may actually have their origin in some other part of the technic or in careless use of the dioxan itself.

TOXICITY OF DIOXAN

Altho the toxicity of dioxan has been mentioned in papers occurring in journals commonly seen by microtechnic workers—Weissberger, Young and Carleton (1934) and Baird (1936)—it seems advisable to discuss the problems at greater length. The most complete pharmacological paper bearing on the subject is that of Navasquez (1935). He reviews the clinical reports on deaths of industrial workers due to dioxan poisoning and also all of the experimental work up to the date of his publication. His experimental work was done on guinea pigs, rabbits and cats. He found that the minimum lethal dose of dioxan for rabbits and cats was 1.5 cc. per kg. when given intravenously, and 2.0 cc. per kg. by stomach tube. Sub-lethal doses resulted in "drunken intoxication" from which recovery was complete and to which an animal's tolerance rapidly increased until even 4.0 cc. per kg. was not lethal. Fairley, Linton and Ford-Moore (1934) showed that of several rats, mice, guinea pigs and rabbits exposed to an atmosphere containing dioxan vapor in proportions of from 1:500 to 1:1000 for from 12 to 202.5 hours only one rabbit died. It was exposed for 69 hours. Most of the other animals withstood even longer exposure. These results, with those of Navasquez, indicate that the toxicity of dioxan for animals is relatively low. While animal toxicity experiments are perhaps not directly transferable to man, these certainly indicate that the toxicity to man must be low and that there is no cumulative effect, but rather the development of a tolerance.

Apparently all the cases of poisoning noted in man followed a period of unusually violent exposure to the vapor and can be considered cases of acute, not chronic, poisoning. The chief pathological lesion was hemorrhagic nephritis resulting from primary necrosis of the intralobular arteries, and the immediate cause of death was acute uremia. Navasquez found no kidney lesions in animals except acute hydropic degeneration of the secreting tubules. He also believes the liver changes described by others are due to glycogen having been dissolved out of the cells and that the liver is uninjured by the poison. In a later paper by Fairley, Linton and Ford-Moore (1936) some evidence is produced to show that the toxic effect of dioxan is due in reality to its oxidation products, oxalic acid and diglycolic acid.

In conclusion it seems safe to say that dioxan is relatively non-toxic to man and laboratory animals. It is certainly wise, however, to keep it covered as much as reasonably convenient and to avoid unnecessary soaking of the hands in it.

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ORSEILLIN BB FOR STAINING FUNGAL ELEMENTS IN SARTORY'S FLUID

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During the past year, students in the senior author's advanced class of mycology have used various stains and technics to bring out the various morphological features in the classes of fungi. The method described has several advantages. First, the process is rapid; second, the details are simple; third, the essential features such as nucleus, spore, and ascus, are made distinct; fourth, plasmolysis does not result; and fifth, ringed slides are permanent.

Orseillin BB¹ (0.25% in 3% glacial acetic acid) similar to that used by Cohen and Doak² was added to Sartory's fluid described by Linder.³ We found that plasmolysis was less likely to result if the amount of phenol crystals was reduced from 20 g. to 10 g. The addition of 1 cc. of the stain to 10 cc. of the mounting medium is ample. Teased sections of fresh, dried, or preserved material, placed directly in the mounting medium to which the stain has been added, will show fine details after approximately 24 hours.

For smaller objects such as nuclei, small spores, etc., teased specimens should be placed directly in the stain for periods varying from one to four minutes. After the excess stain has been washed out and the material has reached the desired depth of staining, clear Sartory's fluid is added and the specimen ringed.

DETAILS OF TECHNIC

Method 1: To a modified Sartory solution (consisting of carbolic acid crystals, 10 g.; lactic acid syrup, 20 g.; glycerin, 40 g.; and distilled water, 20 g.) add 0.25% orseillin BB in 3% glacial acetic acid in the proportion of 1 part to 10 parts.

Fresh, dried, or preserved material teased apart is mounted directly in this solution, and the cover slip ringed with any standard ringing solution.

¹The sample employed in this work was obtained from Dr. G. Grübler & Co., Leipzig.

²Cohen, Isadore and Doak, K. D. 1935. The fixing and staining of *Liriodendron Tulipifera* root tips and their mycorrhizal fungus. *Stain Techn.*, 10, 25.

³Linder, David H. 1929. An ideal mounting medium for mycologists. *Science*, 70, 430.

Method 2: Fresh, dried, or preserved material teased apart, is placed directly on a clean slide and covered with a 0.25% orseillin BB in 3% acetic acid.

Stain from 1 to 4 minutes. It is well to watch this process under the microscope in order to estimate the degree of staining.

Wash off all excess stain with 3% glacial acetic acid in distilled water. Washing should not take more than 5 minutes. In some instances a 1.5% or a 2% solution of the acid may be desirable.

Cover with clear, modified Sartory's fluid.

Add clean cover slip and ring with any standard ringing solution.

PRECISION SECTIONING OF WOOD¹

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ABSTRACT.—In discussing the theories and mechanism of sectioning, it is pointed out that virtually no concrete knowledge exists on the subject. The function of different mechanisms and their role in cutting different types of tissue, and the value of microtome knives versus safety razor blades is discussed. Razor blades failed to cut precise sections; a technic for sharpening a knife to give precise sections is outlined, pointing out errors to avoid in sharpening and sectioning. Various types of knife edges are illustrated by photomicrographs. The procedure of sharpening and sectioning technics for critical results is explained. The mechanism of cutting in wood appeared to be of the crushing and tearing type, indicating the necessity of final polishing in sharpening.

The necessity of minutely exact sectioning to preserve original details of structure is too well realized to require emphasis. Unfortunately, like many common arts when subjected to searching scrutiny, the judicious investigator is compelled to conclude that the mechanism of this type of cutting is obscure. Due to the absence of detailed information and the opposed opinions of well qualified authorities, it appeared to be worth while to report the results of experience in critical sectioning of difficult material, and to present the specific technic and conditions which contributed to successful sectioning. Apparently, in spite of long and intensive use, no publication has ever set forth the exact conditions of blade and edge and their specific and ultimate effects upon woody tissue.

THE MECHANISM OF SECTIONING

Obviously, an understanding of the mechanism of cutting would contribute greatly to the sharpening of the knife and the actual technic of sectioning. It seems to be quite illogical to conclude that cutting is a shearing action in view of the extreme narrowness of the knife edge. Splitting likewise appears to fail to explain the cutting of gel-like materials. Tearing, by microscopic or submicroscopic saw teeth, offers a better explanation for this class of material since it is well known that resilient material like rubber is not cut as easily by a

¹Released as Scientific Journal Series Paper No. 1512 thru the courtesy of the Director, Agricultural Experiment Station, University of Minnesota.

smooth edge as by a jagged edge. Still another explanation is that of 'molecular splitting', i. e., the wedging apart of secondary valence forces. Evidence in favor of this theory is found in the apparently well-known phenomenon of a difference in the static electrical charges of a sectioned non-conductor such as paraffin, while a reversal of the process, causing the same friction but not the splitting, fails to cause the opposing electro-static charges. Crushing, on an extremely fine scale, with subsequent rupture and separation, seems quite plausible, particularly when it is recalled that the extremely small average width of the edge itself would be apt to crush and push aside the tissue on a submicroscopic scale. Beyond these simple theories, it is difficult to offer a convincing explanation of the mechanism involved. In passing, it should be mentioned that the sliding angle and the cutting angle of the knife might change the type of cutting involved, i. e., tearing, splitting, crushing, etc., and that several kinds of cutting might operate simultaneously. Admittedly, such minute changes in structure are of no importance in ordinary sectioning, and it is only in regard to extremely precise sectioning that these remarks are intended to apply, as for example, the study of cell-wall lamellae where slight crushing and pushing aside would confuse or obliterate the true structure.

MICROTOME KNIFE VERSUS RAZOR BLADE

The description of the equipment and manual operations of sectioning, besides being a familiar tool of all microtomists, appears in the literature in comprehensive, detailed, and thoroly excellent accounts, and needs no comment but citation.

Chamberlain (1932) recorded technics for a wide variety of materials. Chamot and Mason (1931) described general sectioning methods with a splendid bibliography of original works. Krause (1927) presented a wonderfully complete and finely illustrated account of successful microtome methods. Gatenby and Cowdry (1928) set forth general histological technics. In the closely related field of textile microscopy, Preston (1933) and Schwarz (1934) described useful sectioning methods. More recently McClung (1937) has presented a comprehensive account of microtechnical methods. Of particular interest with specific reference to wood are the notes of Garland (1935) on the sectioning of woody tissue.

The technic for obtaining and the criteria for judging a critical edge are not so well defined. Even with regard to woody tissue, to which these remarks are exclusively limited, there appears to be at least two antipodal schools of thought in regard to the characters which mark a truly sharp edge, and consequently, opposite views of

the mechanism of sectioning. The older school adheres to the preference for a perfectly smooth and straight edge (even when viewed under fairly high magnification), sharpened by careful grinding, subsequent polishing on rouge, and final polishing on leather. A typical schedule might include initial grinding on a yellow Belgian hone, finer grinding on a blue-green hone, polishing on a red rouge strop, and final polishing on a fine leather strop. This process not infrequently consumes a matter of many hours for a critical edge. Equipment of this sort is designated by these names and is available from any large distributor of microtomes and microtome accessories.

Another group champions the use of the modern safety razor blade, sharpened chiefly by grinding alone, with polishing effects on the knife edge at a minimum. Thus, two opposite types of edges are produced, one smooth and straight, the other scratched and saw-toothed. In defense of the latter, Chamberlain (1932) said in part, "These (specially hardened razor blades) are ideal for histological work" (p. 10), and "... the holder holds Durham Duplex blades very well, and the Gem or Star blade, with the back broken off, is ideal for wood sections. Of course, if one likes to sharpen microtome knives, they are long and will cut while sharp." (p. 141). He also evidently preferred the saw-tooth edge to the smooth one, judging by the predominance of grinding and absence of polishing in his technic: "There should be two good hones: a fine carborundum hone for the preliminary sharpening, and a yellow Belgian hone for finishing. . . . If the second hone be quite hard and the finishing skillfully done, little or no stropping may be necessary." (p. 11). In the author's experience, neither razor blades nor a carborundum stone yielded satisfactory sections of mature wood (which, from air-dry condition, were softened only with water to avoid compositional changes prohibited by the nature of subsequent microchemical studies). A typical safety razor blade edge, showing the scratches and saw-toothed edge, is shown in Fig. 1.

A somewhat similar jagged edge is put on all new microtome knives by a leading manufacturer. The edge is obtained by grinding on a yellow Belgian hone in one direction only, instead of reversing the direction, followed by 6 to 12 strokes on a canvas strop. Such an edge is shown in Fig. 2. The efficacy of sharpening is tested by this manufacturer by cutting sections from an inch square block of paraffin in a rotary microtome. If a 10 μ unsupported section crumples in cutting, the edge is judged unsatisfactory; if the section comes away from the knife in an uncrumpled condition and of approximately the same width as the original paraffin block, the edge is considered satis-

factory. Such an edge may produce satisfactory sections on soft or succulent tissue, but it is particularly to be emphasized that it fails in producing critical sections of harder tissue. In this connection it may be mentioned that the author was unable to duplicate Garland's (1935) successful use of a plate glass disc driven by an electric motor. Also, the recent fabricated stones recommended for microtome knife sharpening failed to produce a successful edge due to the coarse scratches and saw-teeth produced on the knife edge.

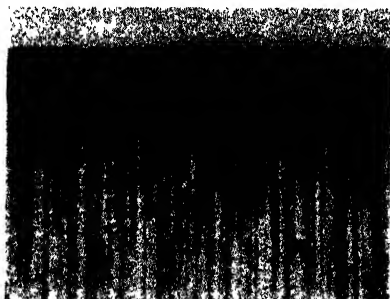


Fig. 1. The edge of a typical safety razor blade with large nicks rather common due to deeper scratches. Note the scratches leading to the extreme edge. $\times 65$.



Fig. 2. The edge a leading manufacturer sends out on all microtome knives. Sharpened on a yellow Belgian hone without reversing direction of strokes. Finished by a few strokes on a canvas strop. Note the jagged edge. $\times 65$.

THE TECHNIC OF SHARPENING

The most successful technic on woody tissue, in the author's experience, is as follows. The knife was preferably a standard microtome knife, of V section, and equipped with a back which was always put on in the same position. The edge was straight with no 'sway-back' on the extreme edge. This condition was obtained by grinding the knife, with the back in place, on plate glass with a fine carborundum powder. The straight edge was then ground on a yellow Belgian hone in the manner described by Krause (1927). When the crossed scratches were uniform over the entire blade and extended completely to the edge (as viewed under the microscope, Fig. 3), the blade was carefully cleaned and the process repeated on the blue-green hone. After the coarse scratches of the Belgian hone had been replaced by the finer ones of the blue-green hone (Fig. 4), the blade was again carefully cleaned, and the stropping begun. The first strop was any microtome knife strop, free from embedded particles of either hone, which was prepared by spreading on a thin film of light mineral oil and then rubbing in a light dust of fine jeweler's rouge

with the finger. The only restriction on stropping was that it had to be uniform over the entire length of the edge; the reversal of the direction of the strokes was not important. An edge partially polished on the rouged strop is shown in Fig. 5. If the strop and rouge surface were in good condition, and the final stropping was done with light strokes, the edge was ready for ordinary use. For precise work, final light stropping on a fine, *clean* strop assured a smooth edge capable of cutting excellent sections.

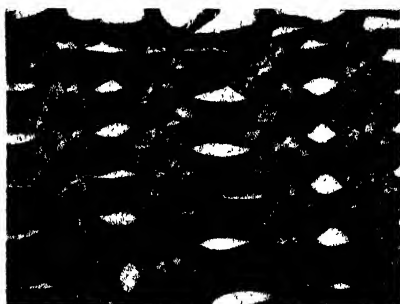
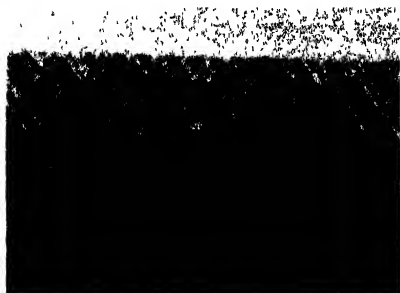


Fig. 3. First step in sharpening; yellow Belgian hone with direction of strokes reversed. $\times 65$.

Fig. 4. The same edge as Fig. 3 except the Belgian hone scratches have been replaced by those of the blue-green hone. $\times 65$.

Fig. 5. The same edge shown in Fig. 4 after partial polishing. The arrow indicates a scratch caused by grit in the strop making a critical edge an impossibility. $\times 165$.

Fig. 6. Section of Douglas fir showing diagonal striae which probably represent crushing and smearing of structure by extremely fine nicks in ordinary knife-sharpening technic. To all appearances this section was ideal before digestion. $\times 220$.

These two stropping operations not infrequently consumed several hours for precise work. It is particularly to be noted that one of the most common causes of failure in the final stages of knife sharpening was the presence of dust and grit on the strops. Further polish-

ing was useless as the grit continued to leave scratches. Apparently, no successful method of removing grit from strops is known. Grit absolutely prohibited precise sectioning results. The final appearance of the critical edge consisted of a perfectly smooth margin of polished metal next to the edge and complete freedom from any irregularities of any kind on the actual edge, when viewed under a magnification of 200 diameters with a single incident beam of light thrown from all azimuths and vertical angles.

Naturally, the hardness of the knife is extremely important on hard tissue; the thin edge bends if the steel is too soft and nicks if too hard. Obviously, hard material to be cut should be softened to a safe margin above the point of plasticity, flowage, or collapse under the knife. One effect of stropping is particularly to be noted: during honing the grinding angle is constant; during stropping it increases progressively. This is due to the sinking of the edge into the soft leather; when the blade passes and the leather springs back to its normal position, it removes more metal from the extreme edge than from the back and center of the ground bevel. Naturally, too steep a 'stropping angle' and consequently 'cutting angle' makes critical sections an impossibility. This effect is easily detected and has the following appearance: The heel of the bevel still has most of the final hone scratches, the central part of the bevel shows moderate to fairly complete obliteration, while the extreme edge shows a smooth surface and freedom from scratches or discontinuities. It follows, therefore, that only a minimum amount of polishing to remove scratches can be allowed, that stropping should consist of *many light* strokes rather than heavy strokes, and that the final hone scratches should be as fine as possible in order to keep the section of the double bevel nearly a true V rather than one with convex sides, resulting in a steep cutting angle. The character of the edge, showing the effects of the yellow Belgian hone, the blue-green hone, and partial stropping are shown in Figs. 3-5, inclusive.

THE TECHNIC OF SECTIONING

The conditions of softening material for sectioning are too well known for discussion, altho microchemical studies usually impose such handicaps to softening procedures as to preclude their use. In general, boiling in water alone is permissible, altho steam, according to the method of Kisser (1926) may sometimes be used. Since generalizations can not be made intelligently, due to the many special conditions, the requirements of the particular problem must be the deciding agent. The cutting angle, i. e., the dihedral angle between the median plane of the knife and the sectioning plane, should be as close

as possible to the final stropping angle to utilize the full cutting efficiency of the knife and to reduce tearing due to shear. The sliding angle, i. e., the angle between the long axis of the knife and the direction of knife movement (on sliding microtomes) should, in general, tend to be small rather than large; in other words, a long portion of the edge should be used for each section. Again, multitudinous hardness and tensile properties of the tissue prohibit exact specifications. This small sliding angle, of course, aggravates the tendency for the section to curl. This may be overcome by either of the common methods: light pressure from the finger or a camel's hair brush.

THE MECHANISM OF SECTIONING

A particularly interesting fact was discovered in an earlier study (Bailey, 1936) bearing on the fine mechanism of sectioning. Blocks of dry mature Douglas fir (*Pseudotsuga tarifolia* Britt.) were boiled in water to immersion and sectioned with a standard microtome knife according to ordinary methods. Under a magnification of 1150 diameters, which approached the limit set by resolution (theoretically, about 1400 diameters for light of 500–600 $m\mu$ wavelength), and even when mounted in media of far different refractive indices, to show and exaggerate conditions on the cut surface, no details of structure or heterogeneity could be detected in the secondary wall itself. When the sections, however, were digested in sulfite liquor used to delignify wood for paper production, and the lignin accordingly removed, the section presented the appearance shown in Fig. 6. Streaks across the section, similar (but much finer) to the crushing streaks by nicks, were plainly visible. This would appear to indicate that a chemically untreated section even under optimum conditions of visibility and resolution, altho apparently a perfect section, may be far from ideal. Also, it follows that this digestive treatment may serve as an extremely delicate test for critical sections. Presumably the digesting liquor removed lignin, probably in non-uniform distribution, leaving a cut surface which was not uniform, and hence, not ideal from the standpoint of a critical study of cell wall lamellae. Further, it points rather definitely to a distinct surface flow of the cell wall with certain portions of the knife edge (similar to the flow of metals in bearings), indicating the danger of over-softening. The obvious conclusion seems to be that in colloidal material like wood, the mechanism of cutting is submicroscopic tearing, crushing, or a combination of both, rather than of the splitting or molecular separation types. If this conclusion be correct, it follows that the ultimate goal of sharpening is to reduce the size of nicks to the point where their effects will be of a submicroscopic order. Polishing, therefore, should be the final step,

with a very fine strop and with very light strokes, as heavy strokes bend the extremely thin edge back and forth until the edge frays and portions break off causing new nicks. If this conclusion be correct, it emphasizes the necessity of using an extremely long portion of the blade in cutting as noted above and recommended by Garland. Finally, the above conclusion seems to be in good agreement with well-known facts: rotary microtomes fail to give precise results due to the absence of sliding action of the edge, crushing and tearing seems to explain the cutting of wood on the same basis as other colloidal materials, and the parallelism of nicks and crushing can be followed visually down to the limit of resolution. It follows, therefore, that the same mechanism should operate immediately below the limit of resolution.

In conclusion it is hoped that this brief communication will aid other investigators in difficult sectioning problems on wood. It is to be noted that the above remarks are intended to apply specifically to the mature woody tissue of the xylem with no intent to decry the equipment or methods which unquestionably are invaluable on other types of tissue.

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MICROSCOPY WITH FLUORESCENT LIGHT

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ABSTRACT.—The principles of fluorescent microscopy are discussed, together with the apparatus necessary for its study. Specially necessary is a lamp giving radiation in the ultraviolet, with filters to remove most of the visible light. Some histological structures have a natural fluorescence and may be studied directly. In other instances fluorescence is induced by the addition of various activating substances (usually dyes) known as fluorochromes. A list of commercial preparations of this sort is given, together with the type of fluorescence which they induce in various histological structures.

It has been known for some time that most living things are profoundly affected by the short light waves beyond the visible spectrum, and much information on their physical makeup has been obtained by observing the appearance of these organisms under the influence of invisible light rays.

The function and composition of organic structures is so complex that it is distinctly worth while to use every available method to bring about a complete understanding of these characteristics. It is certain that ultraviolet radiation not only has a deep physiological effect on animals and plants but it also gives definite characteristic appearances to various structures, and thus throws light on their chemical and physical constitution. The invisible short waves primarily affect the individual cells or structural units, and if these can be studied before, during, and after ultraviolet treatment, one should discover enough of this effect to gain some understanding of the changes which occur in the plant or animal as a whole.

The ultraviolet microscope is, therefore, of much use for physical analysis, for most tissues when excited by the short light waves become clearly visible because they glow with a radiance of their own, or to use a more common term, they fluoresce.

The use of fluorescence in microscopic research is not a recent development, but more general use of fluorescence microscopy has been prevented by the technical difficulty of obtaining a high-intensity concentrated light which is strong in the ultraviolet region. Within the past three or four years, however, an arc lamp¹ with electrodes of a special metal alloy has been perfected, which gives an extremely efficient radiation between 300 and 400 m μ . More re-

¹The Haitinger arc lamp, manufactured by C. Reichert, Vienna.

cently a lamp has been developed which makes use of a mercury vapor arc in a quartz capillary tube. This lamp gives a concentrated light of high intensity and very satisfactory spectral distribution, beginning at about $300\text{ m}\mu$ and extending deep into the infra-red.

This new lamp is very effective and moderate in cost. It may be used freely without any special precautions to shield the eyes from direct or reflected ultraviolet rays, as none of the harmful short waves emanate from the lamp. The protective glass bulb in which the quartz tube is mounted, the condensing lenses, etc., filter out waves shorter than $310\text{ m}\mu$.

APPARATUS REQUIRED FOR FLUORESCENCE MICROSCOPY

Light Source. Arc lamp with metal electrodes, or high-intensity mercury vapor arc lamp. The latter should be used in a housing which permits it to be burned base up. A metal mirror by which the arc may be focussed sharply on the microscope mirror, gives the desired concentration of light. The condensing lens of the lamp must pass the ultraviolet rays between 300 and $400\text{ m}\mu$. The mercury vapor arc operates on alternating current only. The main voltage of 110 – 120 is stepped up thru a transformer to 440 for starting, and as the light reaches its full intensity (in 2 to 3 minutes) the voltage drops to 250 . If alternating current is not available, a small motor-generator may be used to convert the direct current to alternating current at proper voltage.

Microscope Mirror of highly polished or chromium plated metal, or ultraviolet glass. Alzak aluminum is the most effective.

Substage Condenser of quartz or ultraviolet glass, with a darkfield stop. The latter is very necessary not only to obtain images of strong contrast, but the darkfield stop prevents the entry of direct rays into the objective. Ultraviolet light causes a fluorescence of the lens system and the Canada balsam with which the lens elements are mounted, and the resulting foginess would be very disturbing if the direct rays were not excluded.

Object Slides should be of ultraviolet glass. For transmitted light ordinary cover glasses are suitable but with incident light ultraviolet glass cover slips should be used. With immersion lenses a non-fluorescing medium such as sandalwood oil is used. For permanent mounts Canada balsam is not suitable but medicinal mineral oil or glycerin are very satisfactory. The refractive indices of these are 1.48 and 1.47 , respectively, so the optical result is not adversely affected. Cover glasses may be sealed with shellac or beeswax.

Filters. For fluorescence observation it is necessary to filter out practically all the visible light. Schott glass filter U G 2 passes only

a small percentage of violet and red, and filter B G 14 eliminates the extreme red. A copper sulfate solution will serve the same purpose as the latter. The red rays if present are sometimes reflected by the object under observation and may be mistaken for red fluorescence, therefore, it is most desirable to screen them out.

APPLICATIONS OF FLUORESCENCE MICROSCOPY

The usefulness of fluorescence in microscopical research is practically unlimited. In the industrial field might be mentioned studies in textiles and paper, organic and inorganic chemistry, drugs and foods, oils and paints, cellulose and rubber, but of even more importance is the possibility of new discoveries in medical and biological sciences, for instance in the study of carcinogenic substances.

Most readers of this journal are primarily interested in biological research; that subject, therefore, will be treated at greater length.

Perhaps the outstanding benefit of fluorescence microscopy is the ability to examine specimens without staining. To quote an authority on the use of the ultraviolet microscope—²

"Dr. Koehler pointed out many years ago that one advantage of the ultraviolet microscope lay in the fact that organic specimens are differentiated in structure by virtue of the selective absorption which they manifest toward ultraviolet light. . . . Unstained specimens respond under the ultraviolet microscope much as tho they were stained. It is generally recognized that the structure of organic material is apt to be profoundly altered by the treatment in preparing it for microscopic examination. The trend in cytological research appears to be toward the study of living material thus avoiding artifacts induced by fixation, staining and mounting. In the final analysis, biologists are interested in the structure, functions and behavior of the living undisturbed cell."

Nearly all animal cells and tissues contain certain elements which will fluoresce in ultraviolet light. The fluorescence which occurs is characteristic of the fluorescing object and is practically independent of the wave length of the exciting light. The effects obtained without staining are very beautiful and in many cases extend over a considerable portion of the visible spectrum.

The following are a few examples of natural fluorescent colors of animal tissues:

SKIN—Intense blue. Blood vessels appear dark and non-fluorescent.

TEETH—Intense bluish white.'

²Lucas, F. F. 1930. The Architecture of Living Cells. *Proc. Nat. Acad. Sci.*, 16 599-607.

BONES—Light blue.

MUSCLES—Intense light green.

AORTA—Intense light yellow.

LUNG—Dark brown, somewhat greenish. Larger vessels of the bronchi fluoresce light blue.

LIVER—Dark brown, somewhat greenish.

KIDNEY—Dark yellow green.

SPLEEN—Capsule purple, pulp deep brown.

PANCREAS—Excretory portions dark brown, islands of Langerhans red.

THYROID GLAND—Reddish gray.

Connective tissue, elastic tissue, colloidal enclosures in the thyroid, fatty substances and pigments fluoresce in most varied tints. Pigments are easily differentiated—for instance hemoglobin and melanin do not fluoresce, whereas lipofuscin shows a yellowish tint, and the so-called pigment of wear and tear shows different shades from light yellow to brown. Fatty substances of the human body fluoresce quite differently according to their nature; for instance, neutral fats, single refracting fatty granules and double refracting lipoids. Secondary colorations, characterized by lesser intensities of fluorescence, enable one to distinguish the most diverse constituents of tissues, such as neutral fats, nerve boundaries, mucus, etc.

Tissues are best observed in a fresh condition and frozen sections are preferable to avoid embedding processes which may cause structural changes. Paraffin embedded sections may be used after clearing with xylene, but the clearing must be thoro as paraffin fluoresces blue and particles left behind may give a misleading impression. All other embedding methods are unsuitable. Sections should be as thin as possible in order to secure a distinct image, because if there are several superimposed elements of structure the underlying ones may cause a considerable amount of disturbing light diffusion.

Preparations which have a natural fluorescence may be examined without any pre-treatment. The range of fluorescence microscopy, however, has been considerably extended by a process developed by Haitinger in conjunction with Hamperl and Linsbauer, whereby specimens are treated briefly with a sensitizing medium which augments the natural fluorescence. The media are solutions of fluorescent alkaloids known as "fluorochromes." For biological purposes use is made of azo dyes, primulins, auramine, berberine sulfate, chelidonium and rhubarb extracts, etc., in various combinations.

In histological examinations no special preparation is necessary except to harden the sections in a 5% solution of formalin for a period

of five to six hours. All other solutions lessen the fluorescent possibilities. After being fixed in formalin and immersed in the fluoro-chrome for the required length of time, the sections are washed in water and differentiated with alcohol, then embedded in glycerin and the cover glass sealed. In case of too intense fluorescence of the specimen, the section may be cleared by washing in glycerin, water or alcohol.

TABLE 1. ACTION OF FLUOROCROMES.

Fluoro-chrome number	To prepare	Fluorescence color	Immersion period		
			Short		Long 12-15 hours Dilution
			Time	Dilution	
102	*Cell nucleus Protoplasm	gold-yellow light yellow	10 sec.	none	1:500
103	*Cell nucleus Plasma	yellow light yellow	3-5 min.	1:10	1:1000
110	*Fat Cell nucleus Fat cells	Turkish blue yellow-green red	1 min	1:10	1:5000
115	Cell nucleus *Elastic fibers *Collagen fibers	faint blue yellow green yellow	1 min.	none	1:100
116	Elastic tissue *Collagen fibers Muscle cross section	yellow green yellow blue	1 min.	none	1:100
120	Cell nucleus Protoplasm *Nerve tissue	dark red reddish yellowish	1 min.	1:10	1:20
121	Cell nucleus *Nerve tissue Blood vessels in three colors	green blue white yellow blue	3 min.	1:10	1:1000
122	*Nerve tissue Fat	rose blue	5 min.	none	1:10000
123	*Nerve tissue Connective tissue	light blue yellow	1 min.	none	1:100
152	*Plants and textile fibers (silk, linen, wool, etc.)	red and yellow	2-5 min.	none	

Various elements take on fluorescent excitation selectively, some brilliantly and some not so strongly. Thus it is often found that with the use of a single fluoro-chrome, a polychrome effect is secured which

has strength of fluorescence sufficient to give a contrast not customarily seen in ordinary histological staining, and in some cases differentiations are made which are difficult if not impossible with ordinary methods.

The fluorochromes are now made up in various combinations³ suitable for selective excitation in accordance with Table 1. The asterisk indicates the structure which is strongly affected by the fluorochrome indicated.

Altho the weaker solutions require a longer immersion period, they give more uniform results and prevent over-coloring. One should experiment, however, with various dilutions and immersion periods in order to get best results for the particular kind of work which is being carried on.

There are other activating agents which are selective in their action on tissue sections; some of the most useful of these are given in Table 2.

TABLE 2. ACTION OF OTHER ACTIVATING AGENTS

	Cell nucleus	Mucus	Medul- lary layer	Elastic fiber	Col- lagen fiber	Muscle	Fat cells
Auroposphine 1:50000	x	x	x	x	x	x	x
Berberine sulfate 1:500000	x	—	—	x	x	—	—
Rosol red 1:50000	x	—	—	x	x	x	x
Geranine G 1:10000	—	—	x	x	x	x	x
Thiazol yellow G 1:100000	—	—	—	x	x	x	—
Thioflavine S 1:1000000	x	x	x	x	x	x	x

(The structures indicated by x are activated in various colors and degrees of intensity, in the concentrations indicated.)

With these chemicals the immersion period is very short, ranging from 1–60 minutes.

Because of the subtle differences which occur in the fluorescent image, the value of the observation depends largely on the ability of the observer to detect minute color differences, but with a little experience one becomes quickly able to detect these fine variations.

³Obtainable from Pfaltz & Bauer, Inc., Empire State Bldg., New York City. Will probably be available soon from various other sources.

Fluorescence microscopy may be used to great advantage in the observations of living organisms. Frogs and mice for instance may be injected with non-toxic fluorescent materials such as aesculine or uranine and the operation of living organs observed without interference with their structure and functions. Valuable data on the flow of injected substances thru the nervous system may be obtained in the same manner.

The foregoing will immediately suggest many other possibilities of fluorescence microscopy which cannot be detailed here. In the study of plant life for instance this method of microscopic observation will contribute much important knowledge, aside from the added interest afforded by the observation of the astonishingly and remarkably beautiful images of specimens,—“something of an unearthly appearance” to quote one of the pioneers in fluorescence.

It is recommended that the research worker consult the literature which refers to his particular field. The references are voluminous and there are many hundred which may be consulted. These have been assembled and classified by Radley and Grant⁴, which up to now is the most complete resumé on this subject. This line of research, however, offers a wide opportunity for much original work and it is safe to predict that many new and valuable discoveries will make their appearance in the very near future.

⁴Radley, J. A. and Grant, Julius. 1935. Fluorescence Analysis in Ultraviolet Light. Van Nostrand, New York City.

JOHN W. CHURCHMAN

In Memoriam

After an illness of several years Dr. John W. Churchman, who was for some time a member of the Executive Committee of the Commission on Standardization of Biological Stains, died on July 13, 1937, at Amityville, Long Island, at the age of 60 years. It is with great regret that we record his passing.

A graduate of Princeton in 1898, he received his degree of M.D. from Johns Hopkins University in 1902. His original profession was as a surgeon, but of recent years he gave his time almost wholly to scientific research. During his life he held positions on the faculties of Johns Hopkins, Yale and Cornell Universities. At the time when he became incapacitated by his illness, he was professor of experimental therapeutics at Cornell Medical School in New York City.

Dr. Churchman's connection with the Stain Commission began early in the '20's when his work on the bacteriostatic action of dyes and its relation to the Gram stain brought his interests into one of the fields that the Commission was then investigating. He thus became one of the first members of the Stain Commission. In 1929 he was appointed by the American Medical Association as their representative on the Commission and as such was made a member of its Executive Committee. He served in this capacity until his retirement. Altho it has been at least four years since he became inactive, this vacancy on the Executive Committee has never been filled.

Few men in this country have been more interested in the biological applications of dyes than Dr. Churchman during his period of greatest activity. It is, accordingly, a matter of much regret to us that this field was deprived of his services by his long illness which has now ended with his death.

BOOK REVIEWS

LEE, BOLLES. **The Microtometist's Vade-Mecum.** 10th Ed. edited by J. B. Gatenby and T. S. Painter. 6 x 9 in., 784 pp. Cloth. Index. J. & A. Churchill, 40 Gloucester Place, London, Eng. 1937. Price, 30s.

Issued for the first time 50 years ago, Bolles Lee's *Microtometist's Vade-Mecum*, best known of English works on microtechnic, now appears in its 10th edition. Arthur Bolles Lee passed away in 1927 and the 9th edition of the handbook appeared in 1928. Since then a good many changes have taken place in the editing of the book, altho the general plan and arrangement of the material is the same. For the 10th edition, T. S. Painter of Texas University has been made co-editor, and a group of eight new collaborators have helped improve the *Vade-Mecum*. The number of pages in the text has been increased from 637 to 739, mainly by including a new section on plant tissue technic. The following chapters are also new: Dehydration, Preparation of Sections by Freezing Technique, Microchemical Tests, Vital Staining, and two appendixes on various salt solutions and fixatives. A former chapter on Micro-Manipulative Technique is omitted. With the exception of a few sections standing as they were in previous editions, most of the material has been thoroly revised. Some chapters, like those on staining, blood and glands, fatty substances, and tissue culture, have been wholly re-written.—J. A. de Tomasi.

McCLUNG, C. E. **Handbook of Microscopical Technique.** 2nd ed. 6½ x 9½ in., 698 pp. Cloth. 82 illustrations. Index. Paul B. Hoeber, Inc., New York. 1937. \$8.00.

This well-known American book on methods of microtechnic now appears in its second edition. As a result of the coopération of a much enlarged group of contributors (33 in the present edition) the text has been increased by 190 pages. New methods and apparatus are described and many of the technics are brought up to date, altho in some sections (as in the chapter on plant cytology) recent findings seem to have escaped notice. New additions are: the dioxan technic for paraffin sections, a technic for neural terminal buttons, a description of the fused quartz rod for cold illumination; full directions for micro-incineration, and two short accounts on the centrifuge microscope and fluorescence microscopy. The new edition contains about twice as many illustrations as the first, is printed on glazed stock and has been improved in general appearance.—J. A. de Tomasi.

LABORATORY HINTS

FROM THE LITERATURE

A DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS
DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

The abstracts given here are intended primarily for laboratory use; consequently the technic in each instance is given in as much detail as possible.

J. A. de Tomasi

Abstract Editor

MICROSCOPE AND OTHER APPARATUS

FEYEL, P. **Bain-marie à paraffine.** *Bull. d'Histol. Appl.*, **14**, 91-4. 1937.

The conventional paraffin oven is replaced by a rectangular water bath 74 x 33 x 15 cm., made from chromium plated copper sheeting. The front part is a low shelf 6 cm. high by 11 cm. wide, running the entire length of the box. In the rear the remaining 22 cm. of width are taken by the bath proper with its top surface 9 cm. higher than the shelf. The whole is in one piece and filled with hot water. On one end are the thermostat, the water inlet with thermometer, and electric connection leading to 2 rheostats inside. On the other end is the outlet with water level. The top shelf carries 20 sunken cups for glass vessels (7.5 x 4 cm.) arranged in 2 rows; also 6 (6 x 7 cm.), for nickel casseroles with lip and handle — *J. A. de Tomasi*.

MICROTECHNIC IN GENERAL

BARNARD, J. E. and WELCH, F. V. **Fluorescence microscopy with high powers.** *J. Royal Micro. Soc.*, **56**, 361-4. 1936.

A source of light is devised for the purpose of supplying an illuminant of sufficient brilliancy with a high proportion of ultra-violet radiations in relation to the total output of light energy. The light emitted by a high-tension discharge between Mg electrodes is found intense and suitable for high-power microscopy. A triangular glass prism with quartz sides is inserted in the path of the beam between the substage illuminator and the stage. It separates out the required radiation and gives dark-ground illumination. The material is mounted on a quartz slide in a non-fluorescent fluid and covered preferably with a quartz cover glass. — *J. A. de Tomasi*.

SCHNEIDAU, J. D., JR. **Two time saving methods of affixing sections to slides.** *Trans. Amer. Micro. Soc.*, **56**, 258-9. 1937.

To speed up the process of affixing sections to slides, either spread the sections on the surface of a 40°C. water bath and float them onto clean slides without a fixative, or spread on the following dilute fixative solution: 1 egg white; glycerin, 50 cc., sodium salicylate, 1 g., dist. water, 1500 cc. Mix thoroly and filter. Place this solution in a siphon bottle with a pipet tip on the siphon outlet above the work table. Close the siphon with a pinch clamp. Place the dry, clean slides on the table near the outlet of the siphon, run enough solution on the slide to float the sections, spread with heat and allow to dry thoroly. — *Virgene Warbritton*.

ZIRKLE, C. **Aceto-carmin mounting media.** *Science*, **85**, 528. 1937.

A new way is indicated of making Belling's aceto-carmin preparations permanent without impairing simplicity and speed of the original method. Certain inert water soluble materials added to the fixing solution act as mounting media when acetic acid and water evaporate. Two examples of suitable mixtures follow:

(1) Macerate specimen on slide in a drop of Belling's aceto-carmin. Add several drops of: aceto-carmin, 80 cc.; Karo corn syrup (dextrose), 10 cc.; Certo (pectin), 10 cc. Heat as in Belling technic. Squeeze out excess liquid from under the cover slip and dry.

(2) Fixing, staining and mounting are performed in one operation with the following mixture: glacial acetic acid, 50 cc.; water, 50 cc.; glycerin, 1 cc.; gelatin (powder), 10 g.; dextrose, 4 g.; FeCl_3 , 0.05 g.; carmine to saturation. Use it undiluted or diluted with aceto-carmine solution as desired. Dissolve first the gelatin in water, then add all other components. Boil and filter. Such a mount will not be melted by heat.—*J. A. de Tomasi.*

DYES AND THEIR BIOLOGICAL USES

BECK, L. V. and NICHOLS, A. C. Action of fluorescent dyes on *Paramecia*, as affected by pH. *J. Cellular & Comp. Physiol.*, 10, 123-32. 1937.

The comparative toxic (total darkness) and photodynamic effects (in sunlight) on *Paramecia* of a series of basic and acidic fluorescent dyes have been determined, at pH 6.2 and 7.4. Ten of the 12 basic dyes studied (cyanine and acridine groups) were more toxic at pH 7.4 than at 6.2. The other 2 showed no toxic effects in half sat. solution over a 48-hour period at either pH. Seven of 9 basic dyes studied had stronger photodynamic effects at pH 7.4 than at pH 6.2. One (acriflavine) showed practically the same photodynamic action at pH 6.2 as at 7.4 and one, used in relatively high concentration, showed no photodynamic action at either pH over a 3-hour period. It is well known that the penetration into cells of organic compounds is favored, in the case of acidic compounds, by lowering the pH and in the case of basic compounds by raising it. The above findings are therefore interpreted to mean that the greater the concentration of a particular fluorescent dye within a cell the more pronounced are its toxic and photodynamic effects. No correlation was found between penetrability and toxicity or photodynamic action when different dyes were compared with one another.—*Authors' Abstract.*

SCHNEIDAU, J. D., JR. Some notes on the use of acid fuchsin-thionin. *Trans. Amer. Micr. Soc.*, 56, 260-1. 1937.

Thionin, as a counterstain for acid fuchsin, stains the ectoplasm of amoeba, the ectoderm of hydra, and the perisarc of *Obelia* a beautiful blue. The nuclei are usually red and other parts pink. Following the ordinary method of fixation and slide making with Schaudinn's fluid and treatment with iodized alcohol, the essential steps of the staining method are as follows: 1% aq. acid fuchsin, 2-4 min.; 10% aq. phosphomolybdic acid, 1 min.; water, 15 sec. or less; thionin (sat. aq. soln. diluted 1:1 with water), 2-4 min.; dehydrate; clear; mount in balsam or dammar.—*Virgine Warbritton.*

PETRUNKOVITCH, A. On differential staining. *Anat. Record*, 68, 267-80. 1937.

This paper emphasizes the importance of destaining with fluids of proper pH and making up staining solutions at the proper pH to get optimum results. The following points are brought out: (1) Effect of fixation on subsequent staining. (2) Staining a longer time than is necessary for complete staining has little effect. (3) The stain solvent has marked effect. In general aqueous solutions are more selective. (4) pH of staining solutions is of highest importance. Various fixations generally require different pH in staining solutions for optimum results. (5) Concentration of stain, if made up at proper pH, not important if end reaction is permitted to be reached. Amount of dye in a solution affects the pH. (6) Temperature at which staining is done has little effect if constant pH is maintained except when staining is done over 60° C. (7) pH of differentiating fluid has important effect.

The following are among the testing methods given by the author: Use of certified stains, extra care in weighing and measuring, care in quality of dist. water, pH measurement with glass electrode at 25° C., use of similar sections or smears, constant time and temperature in staining. Buffer solutions, N/10 HCl, N/100 HCl, and dist. water were the destaining fluids. Dioxan was used after destaining, then xylene and dammar. In the preparation of each stain the pH of each stock solution was obtained, then a curve was constructed by plotting the volume of N/10 HCl and N/10 NaOH against the pH for a constant volume and concentration of the resulting staining solution. This curve could then be used for quickly making up the stain at any desired pH in which the stain would remain in solution. Next, curves for each stain were constructed showing the

density of staining of the various cell components at all values of pH. These curves vary for the same stain after different fixations. Last, he shows that the capacity of holding a stain in a destaining fluid of a given pH is not of the same order as the staining affinity at that pH. Examples of staining and destaining with acid fuchsin, light green, and orange G are given.

The author then shows that with use of curves mentioned before and knowledge of stain retention, one can do very exact double staining without obtaining intermediate shades of mixtures of the dyes, also that one can make preparations with only such cell structures as he wishes stained. Finally, formulae are given for making up various stains at given pH values for persons not having the necessary equipment to measure the pH of colored liquids, but users are warned about the variation of the staining powders as a source of error—*S. I. Kornhauser*.

T'UNG, T. and ZIA, S. H. Photodynamic action of various dyes on bacteria. *Proc. Soc. Exp. Biol. & Med.*, **36**, 326-30. 1937.

Eight organisms were subjected to ordinary visible light from a Mazda bulb while exposed to eosin, mercurochrome, and trypaflavine. Other dyes were used, but data are not furnished. Eosin was notably more active when exposed to light, trypaflavine next and mercurochrome least. Little or no action was apparent against Gram negative rods, altho a Gram negative coccus proved highly susceptible.—*M. S. Marshall*.

VERNON, A. A. Starfish stains. *Science*, **86**, 64. 1937.

Various dyes are tested out as vital stains to trace migration of starfish. It is found that 1:1,000 solutions are non-toxic and stain in less than 5 min. Of the green dyes, Janus green (Grubler & Co.) and brilliant green (du Pont Co.) take well but do not last. Malachite green (du Pont) stains blue. Light green (Grubler), chrome green C. B. and Erie green W. T. (National Aniline Co.) do not take. Of the red dyes, neutral red (Grubler) stains well and holds, while rhodamine B (du Pont) stains well but fades. Of other dyes used, all made by the National Aniline Company, basic brown stains dark and fades slowly, while crystal and methyl violet fade rapidly. Neutral red is found to be the most satisfactory.—*J. A. de Tomasi*.

ANIMAL MICROTECHNIC

CHRIST, H. G. Untersuchungen über die Speicherung von sauren vitalen Farbstoffen in den Spinalganglienzellen der weissen Maus. *Zts. Anat. u. Entgesch.*, **107**, 83-90. 1937.

White mice are injected with vital dyes approximately every other day. Aqueous solutions of trypan blue (0.5 or 1%) , diamin blue or diamin black (1%) and lithium carmine (2.5%) are used, with intervals of 1-34 days. Of 21 animals, 13 survived. The dyes, except lithium carmine, are observed in the spinal ganglion cells of animals treated for the longer periods of time. Trypan blue gives the most intense stain. The stain is always in a granular form in the cells, but its distribution does not appear to be related to anatomical or functional factors.—*H. A. Davenport*.

CRAMER, W. and HORNING, E. S. Adrenal changes associated with oestrin administration and mammary cancer. *J. Path. & Bact.*, **44**, 633-41. 1937.

Osmic vapor fixation is best for following changes in cortical lipoids, medullary adrenalin, and nuclear changes in the adrenal gland. To conserve osmic acid, fix in 6-7 cc. bottles (internal diameter 1.75 cm., flat-bottomed, ground-glass stopper). Insert into the bottle glass tubing 3 cm. long (internal diameter 0.5 cm.), the upper end of which is covered with gauze, so that the gauze projects about 0.5 cm. from the surface of the liquid. Place gland on gauze; stopper; put in incubator at 37° C for 80 min. Remove gland; place in 80% alcohol; run up thru alcohols to xylene and paraffin; section. Sections may be mounted directly in Canada balsam after removal of paraffin with xylene.

To remove lipoids from cortex in order to distinguish better cortical from medullary cells, place sections in commercial turpentine 30 min. Some pure turpentines do not work. Reduced osmic acid may be removed by bleaching 15 min. in a mixture of 80% alcohol and commercial hydrogen peroxide, 3:1. Preparations can then be stained in the usual manner, iron-alum hematoxylin being very suitable. Several figures, two in color, show effects achieved.—*S. H. Hutner*.

DURAN-REYNALS, F. Localization of foreign proteins and dyes in neoplastic growth. *Proc. Soc. Exp. Biol. & Med.*, **36**, 369-70. 1937.

A study was made of the fixation of "T. 1824," pontamine sky blue, brilliant vital red, vital new red, dianil blue and other dyes in malignant tumors of mice following the injection of a mixture of dye and serum intravenously. Localization was detected in 30 min., with maximum fixation in 12-20 hrs. Healthy tissue stains first, after some days staining occurs in necrotic tissues, altho some dyes do not follow this order. The dyes did not appear to affect the growth.—*M. S. Marshall.*

EMMENS, C. W. The morphology of the nucleus in the salivary glands of four species of *Drosophila*. *Zts. Zellforsch. Mikr. Anat.*, **26**, 1-20. 1937.

A slight modification of Belling's aceto-carminic technic is used in the study of salivary chromosomes. Under a reflux condenser in a waterbath heat a saturated solution of carmine in equal parts of water and glacial acetic acid for 3 hr. Let stand for a month. Stain slides 10-15 min. with the tip of an iron needle immersed in the stain. (Twelve drops of 5% FeCl_3 added to 100 cc. of stain a short time before use may be substituted for the iron needle, but give less uniform results.) Place cover slip and heat slightly.—*V. Warbritton.*

FAUTREZ, J. and LAMBERT, P. P. Une nouvelle méthode de coloration histologique de l'hémoglobine. *Bull. d'Histol. Appl.*, **14**, 29-31. 1937.

This technic represents an attempt to establish a color test for hemoglobin in tissues. It is based on the fact that the isoelectric point of hemoglobin is at a pH considerably higher than for most of the other cellular proteins. The technic follows: Fix in Bouin-Hollande, or neutral formol. Embed in paraffin, cut, run down to water and stain 1-24 hr. in a combination of equal parts of: Grenacher's carmalum (boil 1 hr. 2% carmine in 3% $\text{Al}_2(\text{SO}_4)_3$, K_2SO_4 , 24 H_2O); 1% cyanol; Mac Ilvaine buffer solution at pH 6.4. Rinse thoroly in the buffer solution. Differentiate a few seconds in 70% alcohol until the blue color turns red. Mount in balsam. Hemoglobin takes a blue stain.—*J. A. de Tomasi.*

HERTZOG, A. J. The Paneth cell. *Amer. J. Path.*, **13**, 351-60. 1937.

The Paneth cell of the small intestine contains granules which stain well with acid dyes (particularly Congo red) but not with mucicarmine nor silver salts. Hematoxylin and eosin staining does not give sufficient contrast to show the characteristic granules. Most ordinary fixatives preserve the granules but Bouin's gives the best results.—*H. A. Davenport.*

KIRSCHBAUM, A. and DOWNEY, H. A comparison of some of the methods used in studies of hemopoietic tissues. *Anat. Record*, **68**, 227-31. 1937.

This paper deals almost entirely with the relative merits of embedded, sectioned and stained blood-forming tissues as contrasted with dried imprints of cut surfaces of organs or red bone marrow which are subsequently stained to show blood-forming cells. For the sectioned material, fixation by 10% formalin or Helly's fluid, paraffin or celloidin embedding and staining in Dominici's toluidine blue, eosin-orange G, Maximow's hematoxylin and Azure II-eosin, Giemsa or hematoxylin-eosin are given as regular procedures. For the imprints the following is given:

The cut surface of the tissue is touched lightly to the cover slip or slide which is waved vigorously to insure rapid drying. Cancellous bone, without squeezing to extrude marrow, is touched to the slide for bone marrow imprints. The preparation is then stained like a blood smear. The best results are obtained from Pappenheim's May-Grünwald-Giemsa combination. In this method the slide is flooded for 3 min. with May-Grünwald stain. An equal number of drops of buffered dist. water is then added, the slide being agitated at the same time. The diluted stain is allowed to remain on the slide for 1 min. It is then drained off and the slide covered with Giemsa stain, double strength (2 drops of stock sol. to 1 cc. of dist. water) for 10-12 min., as recommended by Ferrata. This time may be varied according to the material. The preparation is differentiated in dist. water and allowed to dry in the air.

Comparison of the cytological characteristics of cells treated by sectioning and imprint is given and illustrated by drawings and photographs. Special emphasis is placed on nuclear and cell size, the distribution of the chromatin in the nuclei,

and basophilia of the cytoplasm. The conclusion reached is that sectioning is best for cellular orientation, whereas the dry imprint method is recommended for cellular morphology and ease of preparation.—*S. I. Kornhauser.*

SEKI, M. Contribution à l'étude des colorations intra et supra-vitales. X. Signification de la colorabilité des histiocytes et des réticuloendothéliums. *Bull. d'Histol. Appl.*, 14, 114-117. 1937.

During the process of accumulation of colloidal acid substances in the histiocytes and reticulo-endothelial cells, the basic materials of the cytoplasm must play an important role. These can be demonstrated by the following technic: Fix tissue fragments in 2.5% $K_2Cr_2O_7$ in sat. aq. picric acid solution, or in 5% $HgCl_2$ in Muller's solution. Embed in paraffin. Stain sections by the progressive method for 30 min. in a "very thin" solution of methylene blue eosinate buffered at pH 7. Blot and finish drying by agitation. Clear a few minutes in xylene and mount. In preparations of loose connective tissue from regions near the point of injection with trypan blue are to be seen histiocytes and monocytic forms filled with trypan blue; also fibrocytes, and eosinophiles. The quantitative estimation of cytoplasmic staining in these cells can be obtained by comparison with standardized dilutions of eosin and methylene blue.—*J. A. de Tomasi*

SMITH, W. K. and QUIGLEY, B. A new method for the rapid staining of myelin sheaths. *Amer. J. Path.*, 13, 491-5. 1937.

The method can be applied to frozen, paraffin, or celloidin sections. Mordant 15 min. in 4% aq. soln. of $FeNH_4(SO_4)_2 \cdot 12H_2O$. Rinse in 70% alcohol to remove excess mordant. Transfer to the staining solution which contains 1% hematoxylin and 2-3% glacial acetic acid and is prepared from a 10% stock hematoxylin in abs. alcohol. Stain at about 55° C. for 30-60 min.; thin sections may require less time. Place in half-sat. Li_2CO_3 for 5-10 min. Rinse in tap water, counterstain if desired, and follow by graded alcohol, xylene, and balsam.—*H. A. Davenport.*

SZEPSENWOL, J. Technique d'impregnation argentique des fibres nerveuses applicable a des pieces volumineuses ou a des cultures de tissus. *Bull. d'Histol. Appl.*, 14, 168-76. 1937.

This method of Ag impregnation is claimed to yield dependable results with material from all kinds of animals. Type and time of fixation is especially significant. The material is fixed 5-10 days in 10% formol, then 10-20 days in 4% formic acid in 10% formol at 42° C., increasing gradually to 50° C. This double fixation is prescribed for embryonic tissues, while other material may be spared the first step of treatment. Wash 48 hr. in tap and dist. water. Impregnate 1-2 days (2-3 weeks for large specimens) in 1% $AgNO_3$, 8-9 days in 3% $AgNO_3$ in the dark at 33-35° C. Wash quickly in dist. water and soak 2-4 hr. in 1% $AgNO_3$ in 10% NH_4OH . Wash 10-15 min. in dist. water and 12-24 hr. in 20% neutral formol. Wash again 15 min. in dist. water, dehydrate, clear in chloroform or cedar oil and embed.—*J. A. de Tomasi.*

WARBRITTON, V. and MC KENZIE, F. F. The pituitary glands of ewes in various phases of reproduction. *Missouri Agric. Exp. Station Res. Bulletin* 257, May, 1937.

In this extensive study of ewe pituitary glands, Helly's and Bouin's fluids give best cell type differentiation and it is found that the routine alcohol-xylene-paraffin method hardens the tissues. A useful modification calls for the use of a mixture of paraffin, bayberry wax and semi-crude rubber as embedding material in summer months. The dioxan schedule is also followed in obtaining 2-4 μ sections of entire glands.

It is shown by a variety of methods that the main difficulties in staining of pituitary tissue are due to the prevalence of acid dyes in many of the formulae. Frequently also the color combinations do not yield sufficient contrast and the tissue color obtained is not the one anticipated. The following schedule is mostly used: Run down to water; flood with 10% aniline-acid fuchsin (Grubler); heat to steaming; cool 5-10 min.; rinse in dist. water; stain 2-10 min. in Mallory's aniline-blue-orange-G-phosphomolybdic acid. Rinse in water and quickly in 95% alcohol. Dehydrate, clear and mount.—*J. A. de Tomasi.*

MICROÖRGANISMS

LORENZ, W. Eine Schnellbestimmung der Milchkeimzahl mittels Objektträgerkulturen. *Milchw. Forsch.*, 18, 265-79. 1937.

Further studies are reported on the Frost-Clarenburg technic for the bacterial analysis of milk by the slide culture method. A solution of thionin, 1 g., and phenol (crystallized), 2.5 g., in 400 cc. of water, filtered, with acetic acid, 20 g., added to the filtrate, was used to stain the preparations for 5 min. Special precautions to prevent air contamination of the "little plates" were reported.—*H. Macy.*

HISTOCHEMISTRY

CASPERSSON, T. Exposé sur la répartition des acides nucléiques dans le noyau cellulaire. *Bull. d'Histol. Appl.*, 14, 33-43. 1937.

Nucleic acids display a particularly intense absorption of light in certain regions of the ultraviolet spectrum. This absorption depends upon their content of purine and pyrimidine bases and is of much larger order than is the case with albumin. The inference is that, within a cell, a structure containing thymonucleic acid and surrounded by albumins will easily be revealed by a marked light absorption if the wave length of the light corresponds to that of maximum absorption for the acid. As the measuring of absorption can be extended to the limit of the power of definition of the microscope, the study of ultraviolet absorption of cellular elements is carried out by means of Köhler's U. V. photomicrographic apparatus. The object is photographed under a microscope at a number of wave lengths of ultraviolet light. The pictures are taken on special plates under identical conditions. The degree of blackening is then measured with a registering microphotometer or a photoelectric cell. These measurements represent the absorption values of the various structures in the preparation, including those bearing thymonucleic acid combinations.—*J. A. de Tomasi.*

JOYET-LAVERGNE, P. Sur la mise en évidence des zones d'oxydation dans la cellule vivante par la méthode des sels de cobalt. *Compt. Rend. Acad. Sci.*, 204, 1588-90. 1937.

Mount fresh material directly in aq. CoCl_2 1:4000, or in aq. $\text{Co}_2(\text{SO}_4)_3$ 1:3000. Plant material requires a higher concentration (amount not given) and must be colorless. Oxidation is indicated by formation of green salt. Low concentration does not injure living material.—*E. Weier.*

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